Summary of Special Toxicity - see Overall Summary and Evaluation

OVERALL SUMMARY AND EVALUATION

P67.6 is an IgG_4 monoclonal antibody specific for the $CD33^+$ antigen which is a 67 kDa glycoprotein expressed specifically on the surface of early myeloid progenitor cells and leukemic cells of 65-90% of AML patients. The murine P67.6 conjugated to fluorescent reagents is marketed as Anti-Leu-M9 for the detection of $CD33^+$ cells by flow cytometry. $CD33^+$ is not expressed on stem cells, in any other tissue, nor in any species besides humans and the large primates. Neither humanization of the antibody nor conjugation of its lysines to multiple calicheamicin residues with the DMH AcBut linker significantly alter the specificity or affinity of the antibody. The hP67.6 conjugate possesses exceptional cytotoxic specificity (77,000-fold) against $CD33^+$ cells compared to $CD33^-$ cells and is 2,000-fold more potent than unconjugated drug. The calicheamicin residues are released at acidic pHs as is found in lysosomes; disulfide exchange does not appear to be an important release pathway. Studies with the hP67.6 conjugate or with biotinylated hP67.6 did not reveal any adverse reactivities with human tissues that could not be attributed to macrophages/monocytes. Iodinated mP67.6 has been given to humans up to 20 mg/m^2 and selectively targets the bone marrow. Sixteen times the starting human dose of hP67.6 conjugate produced minor changes in blood pressure and cardiac output in dogs. Changes in the EKG and heart rate were noted at 52-times the stating human dose.

The starting dose of 0.25 mg protein/m^2 in humans (6.26 µg/m^2 equivalents of calicheamicin) is below 1/10th of the LD_{10} in rats. Double this dose produced no toxic signs in chimpanzees. hP67.6 did not produce hemolysis in blood. It hydrolyses slowly in buffer solution at physiologic pH and in human plasma. Very little dissociation of calicheamicin from the antibody occurs *in vivo*. Even if all of the calicheamicin groups were to hydrolyze at once upon hP67.6 conjugate administration, the starting calicheamicin equivalents dose of 6.25 µg/m^2 is at least 96-fold less than the LD_{10} of either 184,538 or 191,305 in rats, and is 40-fold less than the dose of 184,538 which when doubled did not kill beagle dogs. The calicheamicin by-product generated after completion of its damaging reactions (190,396) is essentially non-toxic up to 6,000 µg/m^2.

The hP67.6 conjugate is cleared very slowly from the plasma of monkeys (7 days) but more rapidly from rat plasma (3 days). This is consistent with the observation that hP67.6 conjugate is strongly immunogenic in rats, but only weakly so in monkeys. The effect on the pharmacokinetics of the hP67.6 conjugate of having the CD33 antigen present is not known.

When given weekly for 6 doses, the hP67.6 was not lethal to rats up to 7.2 mg/m^2 nor in monkeys up to 21.6 mg/m^2. In rats, gross pathologic changes in the 7.2 mg/m^2 group which did not resolve after 4 weeks of recovery were small testes and pale kidneys. Histopathologic changes at 7.2 mg/m^2 that worsened in the liver during recovery were karyocytomegaly and oval cell/bile duct proliferation; marked changes in the histopathology of the testes, atrophy of the mammary glands, and slight changes in the kidney were also noted that did not resolve during recovery. These changes were consistent with clinical chemistry and hematologic changes indicative of hepato, renal, and hematopoietic toxicities resulting from hP67.6 conjugate administration. In monkeys given multiple doses of hP67.6 conjugate, histopathologic changes at 21.6 mg/m^2 still present after the recovery period were slight tubular basophilia and moderate amount of eosinophilic material in the kidney, slight brown pigmentation of Kupffer cells and moderate single cell hepatocyte necrosis, and atrophy of the lymphoreticular system. These gross and histopathologic changes were consistent with clinical chemistry and hematology changes indicative of mild hepato, renal, and hematopoietic toxicities associated with the drug product. In the clinical trial, a patient will receive at most 3 doses of hP67.6 conjugate and the starting dose of 0.25 mg/m^2 is well below the doses that caused these toxicities in animals.

RECOMMENDATIONS

There are no pharm/tox safety issues and the study may proceed with a starting dose of 0.25 mg/m² as planned.

a) Comments for further studies: none
b) Discussed with Medical Officer: nothing

Draft Letter, Requests for Sponsor
None

/S/

Paul A. Andrews, Ph.D.
Pharmacologist/Toxicologist

12/29/94
Date

cc:
IND ORIG. and Div. File
HFD-150
/JJDeGeorge
/EHenderson
/PZimmerman
/PAAndrews

jjo 2/7/95

Bradley

APR 14 2000

Division of Oncology Drug Products, HFD-150

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Review No. 1

Key words: CMA-676, calicheamicin, $CD33^+$ antigen

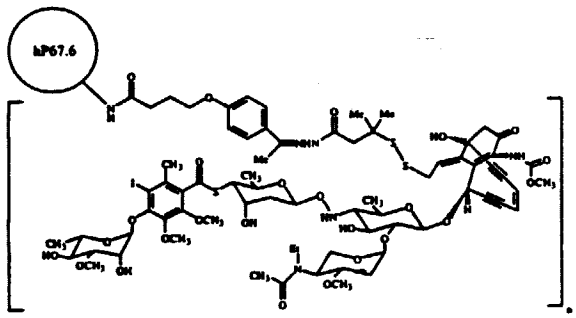
NDA No. 21-174
Serial No(s). 000 **Type:** NDA **Letter Dated:** 10/29/1999 **Received by CDR:** 10/29/1999

Information to be conveyed to the sponsor: No
Reviewer: Sandip K. Roy, Ph.D.
Review Completion Date: 4/14/2000
Sponsor: Wyeth-Ayerst Research
Philadelphia, PA
Manufacturer: Same as above
Drug:

Code Name: CMA-676, CL 555,201
Generic Name: Gemtuzumab Ozogamicin
Proposed Trade Name: Myelotarg
Secondary therapies: none
Chemical Name: hP67.6-NAc-γ_1^I-calicheamicin DMH AcBut conjugate
Other Relevant Names:

184,538	NAc-γ-calicheamicin DMH
191,305	NAc-γ-calicheamicin DMH AcBut
181,441	γ_1^I-calicheamicin
555,001	hP67.6 "naked" antibody
181,287	ε-calicheamicin ("triggered form")
190,396	NAc-ε-calicheamicin

CAS Registry Number: not known
Molecular formula/weight: not known
Structure: The MoAb is a humanized murine monoclonal antibody to human CD33.



n = 0, 1, 2, 3...; average n = 1.9 to 3.1

Related INDs. NDAs, DMFs: IND
Drug Class: Antineoplastic
Indication: Patients with CD33 positive acute myeloid leukemia in first relapse who are 60 years of age or older
Clinical Formulation:

Format	Ingredient	Amount
freeze-dried powder	CMA-676	2.5 mg protein/vial reconstituted to 1 mg protein/ml

Route of administration and dosage form: Each vial contains lyophilized powder containing 5 mg drug conjugate (protein equivalent). Also contains dextran 40, sucrose, sodium chloride, monobasic and dibasic sodium

phosphate. Reconstituted with 5 ml Sterile Water for Injection, USP.

Proposed Clinical Dose: 9 mg/m^2 as a 2-hour intravenous infusion

Previous Review(s), Date(s), and Reviewer(s): IND [redacted] Review #1, 12/29/94, Paul A. Andrews, Ph.D.; IND [redacted] Review # 2, 9/5/96, Paul A. Andrews, Ph.D.

Studies Reviewed in This Submission:

PHARMACOLOGY

GTR-37604 Effects of CMA-676 on Megakaryocytopoiesis in Cell Culture. Vol. 17, p 268.

PHARMACOKINETICS AND TOXICOKINETICS

GTR-30503 CMA-676: Biodistribution and Mass Balance of ^{3}H-CMA-676 Following a Single Intravenous Administration in Rats (Study A9463). Vol. 45, p 141.

GTR-34359 CMA-676: (With Rat Serum Albumin): Intravenous Developmental Toxicity Dose Ranging Study in Gravid Rats: Bioanalytical and Toxicokinetics Report (Protocol 97061). Vol. 45, p 161.

GTR-27093 hP67.6: Pharmacokinetic Comparison in the Monkey of Monoclonal Antibody hP67.6 Derived from Two Different Cell Lines (Protocol 95418). Vol. 45, p 271.

GTR-35324 CMA-676: Characterization of *In vitro* Metabolism of NAc-Gamma-Calicheamicin DMH (CL-184538) and NAc-Gamma-Calicheamicin DMH AcBUT Acid (CL-191305) in Human Liver Microsomes and Cytosol. Vol. 46, p 45.

GTR-35325 CMA-676: Characterization of *In vitro* Metabolism of NAc-Gamma-Calicheamicin DMH (CL-184538) in HL-60 Promyelocytic Leukemia Cells. Vol. 46, p 93.

REPRODUCTIVE TOXICITY

GTR-33829 CMA-676: Intravenous Developmental Toxicity Study in Gravid Rats. Vol. 27, p 1.

GTR-33248 CMA-676 (With Rat Serum Albumin): Intravenous Developmental Toxicity Dose Ranging Study in Gravid Rats. Vol. 27, p 204.

Studies Previously Reviewed (by Paul A. Andrews, Ph.D. in 1994-1996):

PHARMACOLOGY

MIRACL-27251 Binding Characteristics of hP67.6 Antibody and hP67.6 Conjugate To Normal Human Peripheral Blood Leukocytes and Bone Marrow Cells: Comparative Flow Cytometric Analysis. Vol. 16, p 1.

MIRACL-26757 Anti-CD33-N-Ac Gamma Calicheamicin Inhibits Colony 16 384 Forming Activity From Blood and Bone Marrow of Diagnostic AML Patient Samples.

MIRACL-26749 and Report Amendment MIRACL-26900
Evaluation of Hydrazide-, Amide-, and "Hybrid"-Linked 17 35 Conjugates of N-Acetyl Gamma Calicheamicin (CL 181,927) With Murine and Humanized Versions of The Anti-CD33 Antibody, P67.6, Against The HL-60 Tumor As A Model of Acute Myeloid Leukemia (AML).

MIRACL-26753 *In vitro* Cellular Cytotoxicity of NAc-Calicheamicin Gamma (CL 181,927), NAc-Calicheamicin Gamma DMH (CL 184,538), NAc-Calicheamicin Gamma DMH AcBut Acid (CL 191,305) and hP67.6-NAc-Calicheamicin Gamma DMH AcBut Conjugate (CL 555,201). Vol. 16, p 377.

MIRACL-26751 and Report Amendment MIRACL-26899
Synthesis and *In vitro* Cellular Cytotoxicity of Epsilon Calicheamicin CL 181,287 and N-Acetyl Epsilon Calicheamicin CL 190,396. Vol. 16, p 357.

SAFETY PHARMACOLOGY

GTR-35397 Reactivity of Humanized P67 Antibody (CDP77 1) to Normal Human Tissues. Vol. 16, p 172.

GTR-35398 Reactivity of Humanized P67.6 Conjugate (CDP771/CL555,201) to a Panel of Normal Human Tissues. Vol. 16, p 195.

GTR-35399 Reactivity of Humanized P67.6 Antibody (CDP771/CL555,001) to a Panel of Normal Human Tissue. Vol. 16, p 219.

MIRACL-26808 Cross Reactivity of Humanized Monoclonal Antibody hP67.6 with Tissues of Cynomolgus Monkeys and Sprague-Dawley Rats. Vol. 16, p 245.

MIRACL-26816 *In vitro* Blood Compatibility of CL 555,201 (N-Acetyl-Gamma-Dimethyl-Hydrazide-Acetyl-Butyryloxy Derivative of Calicheamicin [CL 191,3051 Conjugated to hP67.6 [CL 555,001]), An Anticancer Agent (Study 94052). Vol. 26, p 117.

MIRACL-26834 Cardiovascular Safety Assessment of CL 555,201 In Conscious Beagle Dogs, Study 94c. Vol. 17, p 112.

MIRACL-26755 Reaction of N-Ac Gamma Calicheamicin DMH (CL 184,538) With Reduced Glutathione. Vol. 16, p 121.

MIRACL-26756 Hydrolysis of hP67.6-N-Ac Gamma Calicheamicin DMH AcBut Conjugate (CL 555,201) In Buffer At Physiologically Relevant pH's. Vol. 16, p 67.

PHARMACOKINETICS AND TOXICOKINETICS

MIRACL-26888 Pharmacokinetics of the hP67.6/3H-N-Acetyl-γ-DMH-AcBut Calicheamicin Conjugate (CL 555,201) Following a Single Intravenous Administration in Rats. Vol. 45, p 94.

MIRACL-26887 Pharmacokinetics of the hP67.6/3H-N-Acetyl-γ-DMH-AcBut Calicheamicin Conjugate (CL 555,201) Following a Single Intravenous Administration in Cynomolgus Monkeys. Vol. 45, p 223.

GTR-36334 CMA-676: A Single Dose Intravenous Toxicity Study of CL-191305 (N-Acetyl-Gamma-Dimethyl-Hydrazide-AcBut Derivative of Calicheamicin), an Anticancer Agent, in Rats: Toxicokinetics Report (Cyanamid Protocol 93026). Vol. 46, p 6.

GTR-36332 CMA-676: A Single Dose Intravenous Toxicity Study of CL-184,538 N-Acetyl-Gamma-Dimethyl-Hydrazide Derivative of Calicheamicin), an Anticancer Agent, in Rats: Toxicokinetics Report (Cyanamid Protocol 92044). Vol. 46, p 18.

GTR-36333 CMA-676: A Single Dose Intravenous Toxicity Study of CL-184,538 N-Acetyl-Gamma-Dimethyl-Hydrazide Derivative of Calicheamicin), an Anticancer Agent, in Dogs: Toxicokinetics Report (Cyanamid Protocol 92047). Vol. 46, p 30.

TOXICOLOGY

MIRACL-26631 A Single Dose Intravenous Toxicity Study of CL 555,201 (N-Acetyl-Gamma-Dimethyl-Hydrazide -Acetyl-Butyryloxy Derivative of Calicheamicin [CL 191,3051 Conjugated to hP67.6 [CL 555,001]), An Anticancer Agent, in Rats (Study 93119). Vol. 18, p 1.

MIRACL-26711 An Exploratory Single Dose Intravenous Tolerance Study of CL 555,201 (N-Acetyl-Gamma-Dimethyl-Hydrazide-Acetyl-Butyryloxy Derivative of Calicheamicin [CL 191,305] Conjugated to hP6.76 [CL 555,001]), an anticancer agent, in rats (Study 94033). Vol. 19, p 1.

MIRACL-26710 A single dose intravenous toxicity study of CL 555,201 (N-acetyl-gamma-dimethyl –hydrazide-acetyl-butyryloxy derivative of calicheamicin [CL 191,3051 conjugated to hP67.6 [CL 555,0011), an anticancer agent, in male monkeys (Study 93214). Vol. 20, p 1.

MIRACL-26817 An Exploratory Single Dose Intravenous Tolerance Study of CL 555,201 (N-Acetyl-Gamma-Dimethyl-Hydrazide-Acetyl-Butyryloxy Derivative of Calicheamicin [CL 191,3051 Conjugated to hP67.6 [CL 555,001]), An Anticancer Agent, in the Chimpanzee (Study 94118). Vol. 26, p 56.

MIRACL-26709 A Single Dose Intravenous Toxicity Study of CL 191,305 (N-Acetyl-Gamma-Dimethyl-Hydrazide AcBut Derivative of Calicheamicin), An Anticancer Agent, in Rats (Study 93026). Vol. 28, p 1.

MIRACL-26628 A Single Dose Intravenous Toxicity Study of CL 184,538 (N-Acetyl-Gamma-Dimethyl-Hydrazide Derivative of Calicheamicin), An Anticancer Agent, in Rats (Study 92044). Vol. 30, p 1.

MIRACL-25706 A Single Dose Intravenous Toxicity Study of CL 190,396 (N-Acetyl-Epsilon Derivative of Calicheamicin), An Anticancer Agent, in Rats (Study 92045). Vol. 32, p 1.

MIRACL-26629 A Single Dose Intravenous Toxicity Study of CL 184,538 (N-Acetyl-Gamma-Dimethyl-Hydrazide Derivative of Calicheamicin), An Anticancer Agent, in Dogs (Study 92047). Vol. 31, p 1.

MIRACL-26813 Six Cycle Intravenous Toxicity Study of CL 555,201 (N-Acetyl-Gamma-Dimethyl- Hydrazide-Acetyl-Butyryloxy Derivative of Calicheamicin [CL 191,3051 Conjugated to hP67.6 [CL 555,001]), An Anticancer Agent, in Rats Followed by a Four Week Recovery Period (Study 94034). Vol. 20, p 61.

MIRACL-26812 A Six Cycle Intravenous Toxicity Study of CL 555,201 (N-Acetyl-Gamma-Dimethyl-Hydrazide-Acetyl-Butyryloxy Derivative of Calicheamicin [CL 191,3051 Conjugated to hP67.6 [CL 555,00 1]), An Anticancer Agent, in Monkeys Followed by a Four Week Recovery Period (Study 94001). Vol. 23, p 1.

GENETIC TOXICOLOGY

GTR-28070 Mutagenicity Test on CMA-676/Calicheamicin in an *In vivo* Mouse Micronucleus Assay. Vol. 27, p 330.

Studies not Reviewed in this Submission:

GTR-36629 HL-60 Binding and Internalization of Radioiodinated P67.6. Vol. 16, p 41.

GTR-36630 The Mechanism of P67 Antibody Internalization into HL-60 Promyelocytic Leukemia Cells Observed by Immunoelectron Microscopy. Vol. 16, p 55.

MIRACL-26754 Evaluation of Relative Immunoaffinities of Four Batches of hP67.6-N-Acetyl Gamma Calicheamicin Dimethyl AcBut (CL 555,201). Vol. 16, p 62.

MIRACL-21788 The Disulfide Calicheamicins. Vol. 16, p 79.

MIRACL-26750 The Glutathione Disulfide of Calicheamicin Gamma and Related Disulfides. Vol. 16, p 133.

MIRACL-26830 Intrinsic Cellular Determination of Apoptotic Versus Differentiative Response of Neuroblastoma Cells To Enediyne Treatment. Vol. 16, p 144.

GTR-31510 A Potent, Selective, Hydrolytically Stable, Anti-PEM Immunoconjugate of Calicheamicin for Solid Tumor Therapy. Vol. 16, p 147.

GTR-35400 Reactivity of Humanized P67.6 and hP67.6-Calicheamicin Conjugate (CDP771/2) to a Panel of Normal Human Tissues. Equivalence Study for New Celline (KD1). Vol. 16, p 172.

GTR-37661 Calicheamicin-Conjugated Humanized Anti-CD33 Monoclonal Antibody (CMA-676) Shows Non-Apoptotic Cytocidal Effect on CD33-Positive Leukemia Cell Lines, But is Inactive on P-Glycoprotein Expressing Sublines. Vol. 16, p 299.

GTR-34019 The Humanized Anti-CD33 Antibody P67.6 Exhibits Minimal Inhibition of Colony Forming Activity from Blood and Bone Marrow of Diagnostic AML Patient Samples (Report from Fred Hutchinson Cancer Research Center). Vol. 16, p 337.

GTR-34020 An Assessment of the Cytotoxicity of hP67.6 Antibody. Vol. 16, p 349.

GTR-36631 Inherent Sensitivity of HL-60, Raji, and MX-1 Cells to Calicheamicin Derivatives using Radiolabeled Compounds. Vol. 16, p 365.

MIRACL-90464 The Preparation and Characterization of Monoclonal Antibody Conjugates of the Calicheamicins: A Novel Family of Antitumor Antibiotics. Vol. 17, p 1.

MIRACL-26752 Variation of Disulfide Stability for CT-M-01-Calicheamicin Hydrazide Conjugates. Vol. 17, p 35.

MIRACL-26747 The Preparation and Characterization of Hybrid Conjugates Prepared from the Calicheamicins and Monoclonal Antibodies. Vol. 17, p 66.

GTR-37315 General Pharmacology Study of CMA-676. Vol. 17, p 184.

GTR-37316 Development of Experimental Method for Detection of Urine Volume and Urinary Electrolytes in Mice and Determination of the Effect of CMA-676 in the Developed Experimental System. Vol. 17, p 246.

GTR-27677 CMA-676: Six Cycle Intravenous Comparative Toxicity Study in Male Monkeys. Vol. 25, p 1.

GTR-33261 CMA-676 (With Rabbit Serum Albumin): Multiple Dose (13 Day) Intravenous Tolerability Study In Female (Non-Gravid) Rabbits With Two-Week Post-Dosing Observations. Vol. 26, p 1.

MIRACL-24627 A Single Intravenous Dose Exploratory Study of Calicheamicin (CAL) Analogs (Antitumor Antibiotics) in Mice (Study 90126). Vol. 29, p 1.

MIRACL-26826 Amendment I To Report 26629. A Single Dose Intravenous Toxicity Study of CL 184,538, An Anticancer Agent, In Dogs (Study 92047). To Explain Why Stability and Concentration of I Microgram/mL Dosing Formulation Were Not Determined. Vol. 31, p 403.

MIRACL-26825 Amendment I To Report 25706. A Single Dose 32 394 Intravenous Toxicity Study of CL 190,396, An Anticancer Agent, In Rats (Study 92045). To Provide Additional Information on the Specificity of ELISA Method of Detection for Calicheamicin Derivatives in Plasma. Statements Re: Toxicokinetics Have Been Revised. Vol. 32, p 394.

MIRACL-25707 A Single Dose Intravenous Toxicity Study of CL 190,396 (N-Acetyl -Epsilon Derivative of Calicheamicin), An Anticancer Agent, in Dogs (Study 92049). Vol. 33, p 1.

MIRACL-26827 Amendment I To Report 25707. A Single Dose Intravenous Toxicity Study of CL 190,396, An Anticancer Agent, In Dogs (Study 92049). To Provide Additional Information on the Specificity of ELISA Method for Detection of Calicheamicin Derivative in Plasma. Statements Re: Toxicokinetics Have Been Revised. Vol. 33, p 337.

MIRACL-19885 An Acute Intravenous Toxicity Study of E33288 Gamma-l-I in Mice (Study 86153). Vol. 34, p 1.

MIRACL-24556 An Acute Intravenous Toxicity Study of E33288-Gamma-1-I (CL 181,441) in Mice with a Five Week Observation Period (Study 86204). Vol. 34, p 39.

MIRACL-24559 An Acute Intravenous Toxicity Study of E33288-Gamma-1-I in Rats (Study 86283). Vol. 34, p 145.

MIRACL-24564 A Comparison Study of the Hepatic Effects of E33288-Gamma-l-I (CL 181,441) in Rats (Study 88025). Vol. 34, p 247.

MIRACL-24563 An Acute Intravenous Toxicity Study of E33288-Gamma-1-I (CL 181,44 1) in Cynomolgus Monkeys (Study 88024). Vol. 35, p 247.

MIRACL-24561 A Single-Dose Topical and Subcutaneous Study of E33288-Gamma-1-I in Rats, with a 120 Day Observation period (Study 87141). Vol. 36, p 1.

MIRACL-24562 A Single Dose Intranasal Study of E33288-Gamma-1-I In Rats, With A 120 Day Observation Period. Final Report. Study No. 87142. Vol. 37, p 101.

MIRACL-24557 An Acute Dermal Toxicity Study of E33288-Gamma-1-1 (CL 181,441) in Rats (Study 86205). Vol. 37, p 196.

MIRACL-26630 A Six Week Intravenous Toxicity Study of CL 184,538 (N-Acetyl-Gamma-Dimethyl-Hydrazide Derivative of Calicheamicin), An Anticancer Agent, in Rats (Study 92080). Vol. 38, p 1.

MIRACL-26707 A Six Week Intravenous Toxicity Study of CL 184,538 (N-Acetyl-Gamma-Dimethyl- Hydrazide Derivative of Calicheamicin), An Anticancer Agent, in Dogs (92081). Vol. 40, p 1.

MIRACL-24625 A 5 Day Intravenous Toxicity Study of E33288-Gamma-1-1 in Rats with An Observation Period of Several Months (Study 87136). Vol. 42, p 1.

MIRACL-24560 A 5 Cycle (21 Day Interval) Intravenous Toxicity Study of E33288-Gamma-l-I in Rats (Study 87137). Vol. 44, p 1.

MIRACL-24484 Magnetic Resonance Imaging (MRI) Study on Rats Dosed with E33288 (CL 181,441, Calicheamicin) (Study 88077). Vol. 45, p 1.

MIRACL-26333 The Effects of the Derivatives of Gamma Calicheamicin, N-Acetyl Gamma Calicheamicin (CL 181,927), N-Acetyl Dimethyl Hydrazide, (CL 184,538), N-Acetyl Dimethyl Acid (CL 186,760)

and N-Acetyl Epsilon (CL 190,396) on Murine Bone Marrow Hematopoietic Colony Formation *In Vitro* (Study 92166). Vol. 45, p 15.

MIRACL-26331 Effects of Gamma Calicheamicin, CL 181,441 on 45 33 Murine Bone Marrow Hematopoietic Colony Formation *In vitro* (Study 92119). Vol. 45, p 33.

MIRACL-26809 Exploratory Studies on the *In vitro* Toxicity of Gamma Calicheamicin (CL 181,441), NAc-Gamma Calicheamicin (CL 181,927), NAc-gamma calicheamicin DMA (CL 186,760), NAc-Gamma Calicheamicin DMH (CL 184,538), and NAc-Epsilon Calicheamicin (CL 190,396) (Study 10192). Vol. 45, p 45.

Note: portions of this review were excerpted directly from the sponsor's submission

PHARMACOLOGY

GTR-37604 **Effects of CMA-676 on Megakaryocytopoiesis in Cell Culture. Vol. 17, p 268.** Conducted by [redacted]

Date of Study Initiation: January, 1999

Objective: To evaluate the effects of CMA-676 on normal human megakaryocyte differentiation with the goal of understanding why some acute myeloid leukemia (AML) patients in the ongoing Phase II clinical trial experienced prolonged thrombocytopenia and delayed recovery of platelets.

Methods:

Liquid Culture Assays
Bone marrow was obtained as Ficoll separated MNC from normal healthy donors. $CD34^+$ cells were isolated using a MACS bead separation system and were treated immediately with CMA-676 or a control antibody conjugate CTM01-NAcγAcBut, (humanized anti-Muc-1 antibody conjugated with calicheamicin) for two hours, washed thoroughly, and placed in liquid culture in X-vivo 10, 0.5% FCS, steel factor (SF), TPO and interleukin-11 (IL-11), a cytokine cocktail shown to promote maximal growth of megakaryocytes *in vitro*. The final conc. of CMA-676 conjugate in the liquid cultures ranged from 0.002 to 0.5 µg/ml. After 14 days in culture, the total number of megakaryocytes were quantitated by flow cytometry by measuring the percentage of $CD41a^+CD14^-$ cells in the cultures.

HL-60 Cytotoxicity Assays
HL-60 (promyelocytic leukemia) cytotoxicity assays were carried out to confirm that the CMA-676 used in these experiments was fully active. Cells were incubated with the conjugate for 24 hours in CO_2 incubator before the addition of ^{3}H-thymidine. Then the plates were incubated for 16 hrs before harvesting. The radioactivity of incorporated [^{3}H]-thymidine was measured by liquid scintillation method. Death of cells is measured by the inhibition of incorporation of [^{3}H]-thymidine during the last 16 hrs of culture. Some cytotoxicity experiments were carried out using human erythroid leukemia (HEL) cells that do not respond to CMA-676.

CFU-Meg Assays
$CD34^+$ bone marrow cells were sorted to isolate $CD41a^-$, CD41a bright and CD41a dull cells. Cells were treated with a range of concentrations of CMA-676 from 0.75 to 10-µg/ml or 0.5 to 10 µg/ml then plated in CFU-Meg assays. Slides were stained and scored after 12 days. Megakaryocyte colonies were enumerated for each progenitor subset.

CFU-C Assays
Following incubation with CMA-676, $CD34^+$ cells were washed and were plated at 3×10^4 cells/mL and incubated in a fully humidified atmosphere for 12 days. Assays were scored by counting colonies using an Olympus inverted phase-contrast microscope. A minimum of 8 fields/well were scored and the mean was calculated. Colony numbers were reported as percent of the media only control. For time course experiments, cells were incubated with CMA-676 at 2 µg/ml or control for 2, 6, 11, or 24 hrs.

Antibody Binding
Biotinylated hP67.6 was bound to $CD34^+$ cells at sub-saturation levels and then stained with Streptavidin-Fitc and a commercially available anti-CD33 labeled with PE. The double-labeled cells were analyzed by flow cytometry. To investigate the binding of hCTM01 to the target cell populations, normal bone marrow cells were labeled with biotinylated hCTM01.

GeneChip Expression Studies
HL-60 and HEL cells were grown to confluence, harvested and washed once in cold phosphate buffered saline. Total RNA was extracted and PolyA positive mRNA was isolated followed by amplification and labeling of the RNA for use on Affymetrix GeneChip oligonucleotide arrays. Double stranded cDNA was synthesized with an oligo-dT primer that contains the promoter site for T7 RNA polymerase. The cDNA was then used as the template for T7 RNA polymerase in an *in vitro* transcription reaction in the presence of biotinylated ribonucleotides. The final antisense RNA product was fragmented to approximately 50 bases in length and was hybridized to the GeneChip arrays overnight. Also

included in the hybridization solution was a pool of 11 labeled RNA transcripts of known concentration. Following hybridization, the arrays were washed and stained with a streptavidin-PE conjugate were scanned on a Hewlett-3. 1.

GeneChip Expression Studies on Normal Bone Marrow
$CD34^+$ cells were selected from MNC obtained from 5 different bone marrow donors, who were normal volunteers. $5 X 10^6$ cells or more were removed from the selected populations and immediately used to prepare RNA for Genechip analysis as described above. The remaining cells were cultured overnight then treated with CMA-676 over a range of concentrations from 0.5 to 10 µg/ml. Cells were washed then plated described earlier.

Results:

Since $CD34^+$ defines a broad population of stem and progenitor cells, the sponsor thought it was important to determine the extent of CD33 expression in this target population. Flow cytometry staining pattern (figure submitted but not shown here) indicated that a large percentage cells (>90%) are positive for the CD33 antigen with a wide variety of staining intensity, indicative of antigen density.

The bone marrow cells in liquid culture were completely resistant to the cytotoxic effects of CMA-676 at concentrations ranging from 0.002 to 0.5 µg/ml, under growth conditions optimized for growth of megakaryocytes. Pharmacokinetic data from the Phase II AML clinical trial revealed higher serum levels of CMA-676 (>1 µg/ml after 24 hrs treatment on average). Consequently, subsequent experiments were carried out with 0.5 – 10 µg/ml CMA-676.

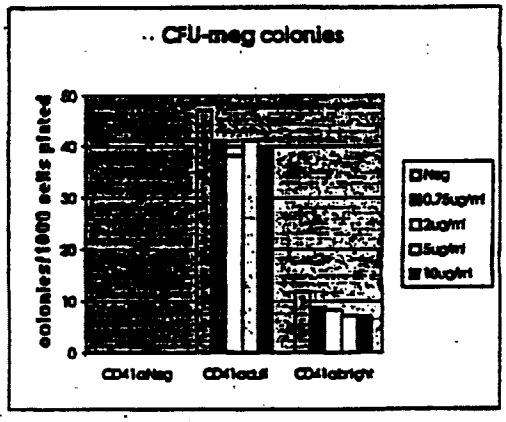
HEL cells are highly resistant to CMA-676 although they are 100% positive for CD33, while HL-60 with slightly lower CD33 expression is extremely sensitive to the drug's cytotoxic effects. This suggests that CMA-676 cytotoxicity does not correlate with the amount of surface expression of CD33 and that CD33 expression alone is not sufficient to explain the sensitivity to the cytotoxic conjugate, CMA-676. To address the differences in sensitivity of HL-60 and HEL to CMA-676, an RNA expression analysis was carried out using Affymetrix GeneChips. The data was filtered for a small subset of genes that may be involved in the cellular response to CMA-676, such as glutathione related genes and multi-drug resistance genes (MDR). Different isoforms of glutathione S-transferase genes showed a range of changes from 4-fold increase in human glutathione S-transferase GSTM4 to 9-fold decrease in glutathione S-transferase pi or GSTP1 (see table below). The sponsor did not attempt to use the GeneChip data to explain the difference in sensitivity of these cell lines to CMA-676.

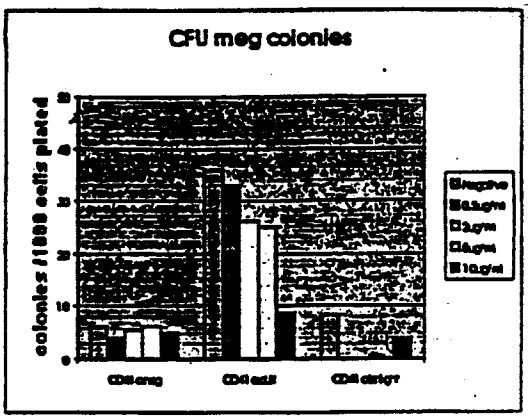
GeneChip™ Comparison Between HL-60 and HEL Cells

			HL60		HEL	
Gene Family	Genbank ID	Description	Decision	mRNA molecules per million	Decision	mRNA molecules per million
Glutathione metabolism	M60233	Human glutathione transferase class mu isoform 4 (GSTM4) gene	Absent	14	Present	53
	D54483	Human glutathione synthetase mRNA	Absent	22	Present	87
	M24485	Homo sapiens (clone pHGST-pi) glutathione S-transferase pi (GSTP1) gene	Present	757	Present	86
	U68520	Human glutathione transferase zeta 1 (GSTZ1) mRNA	Absent	34	Present	50
	J77664	Human placental glutathione S-transferase (GST-II) mRNA	Present	183	Present	141
	U66313	Human glutathione-s-transferase homolog mRNA	Present	83	Present	50
	L08321	Human glutathione S-transferase (GSTM5) mRNA	Present	30	Absent	21
Multiple Drug Resistance genes	M14758	Human P-glycoprotein (MDR1) mRNA	Absent	13	Present	19
	X56723	Human MDR1 (multidrug resistance) gene for P-glycoprotein	Present	18	Absent	15
Beta Actin genes	M10277	Human cytoplasmic beta-actin gene	Present	749	Present	663
	X00351	Human mRNA for beta-actin	Present	1072	Present	894

Genes are listed by Genbank identification number with the Genbank description. The Decision (fourth and sixth columns) indicates whether or not the transcript was detected by the data reduction software (present or absent) in each sample. This decision is made based on the comparison of specific to nonspecific probes. Any member of the three families, which was detected as present in at least one of the samples, was included in the table. RNA concentration is expressed as mRNA molecules per million (fifth and seventh columns). This value is calculated from an internal standard curve consisting of 11 transcripts of known concentration compared to their specific fluorescent intensity. With the Human 6800FL array and current protocols, changes larger than 4-fold are highly reproducible (data is bolded).

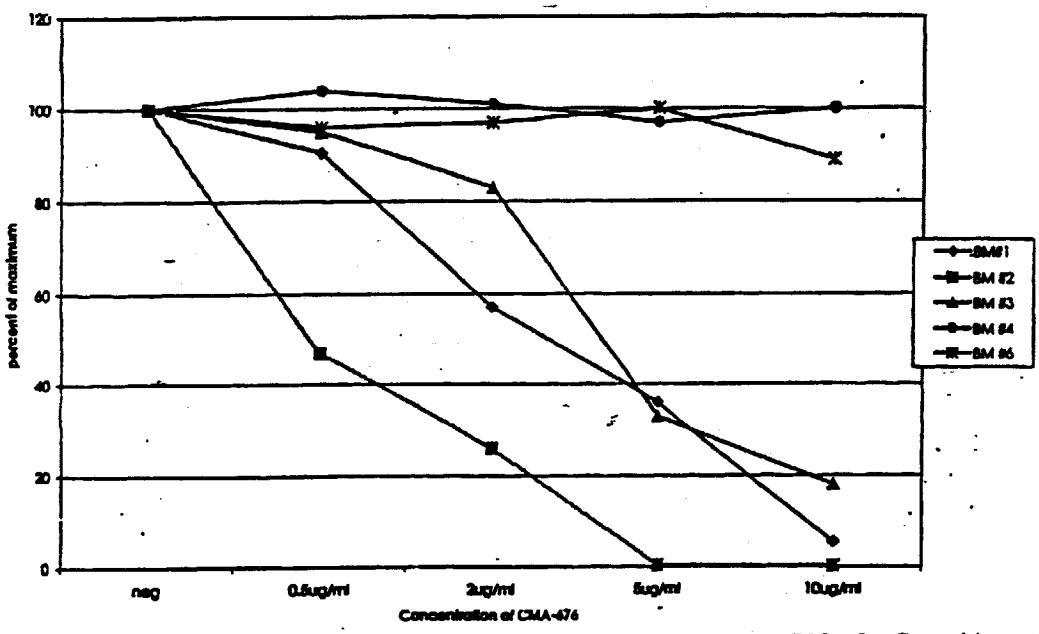
To determine whether subsets of megakaryocyte progenitor cells had variable sensitivities to CMA-676, CFU-Meg assays were set up using $CD34^+$ cells from two different bone marrow samples that had been sorted into CD41a negative, CD41a dull, and CD41a bright. In the first bone marrow sample, the CD41a negative cell population was completely devoid of CFU-Meg progenitors. In addition, all the progenitors in the first bone marrow were highly resistant to the cytotoxic effects of CMA-676, whereas only the CD41a negative cells in the second bone marrow were resistant. The sponsor concluded that because the extent of growth from these progenitors is so small, it is unlikely that these cells contribute extensively to platelet repopulation in patients.





0.5, 2, and 5 µg/mL points not done for CD41a bright cells.

Correlation between gene expression as determined by GeneChip analysis and sensitivity of the same donor bone marrow to CMA-676 was investigated in $CD34^+$ cells selected from 5 normal volunteer donors. As previously observed with other bone marrow donors, sensitivity of these donors to drug treatment was highly variable (see figure below).



The same bone marrow samples from 5 donors shown above were used to prepare RNA for Genechip analysis. Over 3700 genes were detectable in at least one of the five samples on the Affymetrix HuGeneFl microarray. Glutathione pathway is believed to be involved in the conversion of calicheamicin to its most cytotoxic form. However, no significant differences in glutathione pathway mRNAs were observed between these donors which might explain the difference in sensitivity to CMA-676 between these donors. MDR1 (multiple drug resistance) genes, another group of genes known to be involved in resistance to drug therapy is represented twice on the HuGeneFL microarray. However, MDR1 was not detected with either probe set in any of the samples (see table below). Thirty-six transcripts were detected which did correlate with sensitivity to CMA-676. Upon request, the

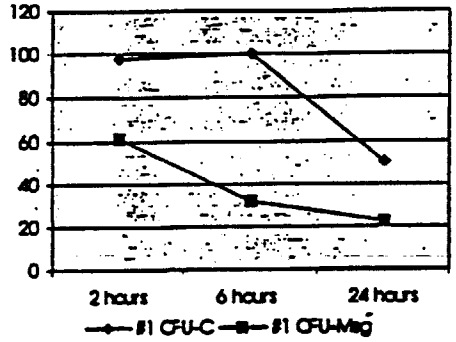
Cytotoxic effects of CMA-676 on formation of total CFU-C composed largely of granulocyte (G), macrophage (M) and granulocyte/macrophage (GM) colonies and total CFU-Meg composed mainly of pure and mixed CFU-Meg colonies were measured at a dose range (0.5 – 10 μg/ml) more comparable to levels in the serum of the AML patients in the Phase II trial. The results of these experiments are summarized as follows:

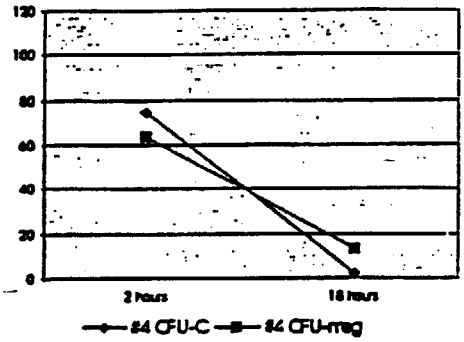
Summary of IC_{50} data of five different bone marrows after treatment with CMA-676

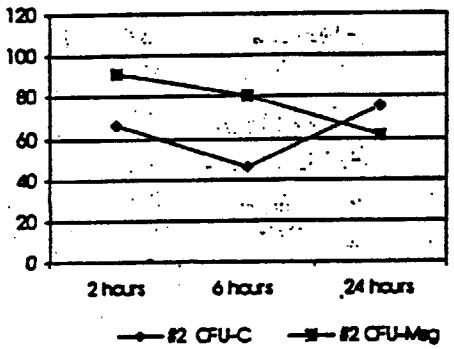
Bone Marrow #	CFU-C (μg/ml)	CFU-Meg (μg/ml)
1	>10	1
2	>10	>10
3	1	1
4	5	1
5	8	10

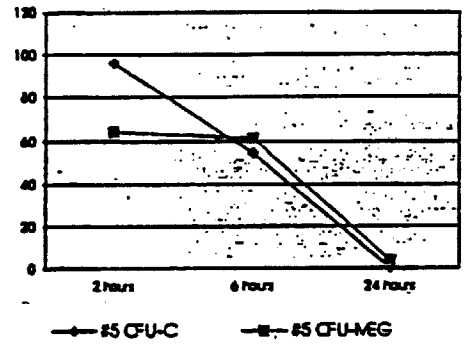
IC_{50}: Concentration of CMA-676 necessary to inhibit 50% colony formation

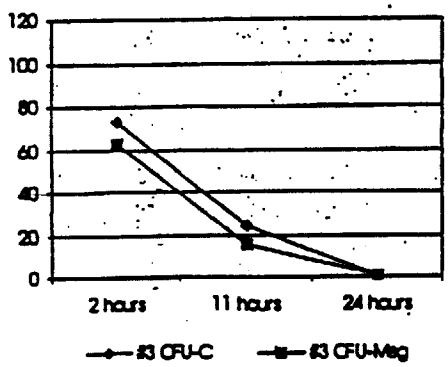
To determine whether the effects of CMA-676 were cumulative with prolonged exposure, cells were treated for various time intervals before plating in colony assays. In some donors, cytotoxicity was evident after only 2 hrs of CMA-676 exposure, yet in other donors there was a marked increase in sensitivity of progenitors with increased exposure time. The sensitivity of CFU-C and CFU-Meg progenitors was also variable (see figure below).

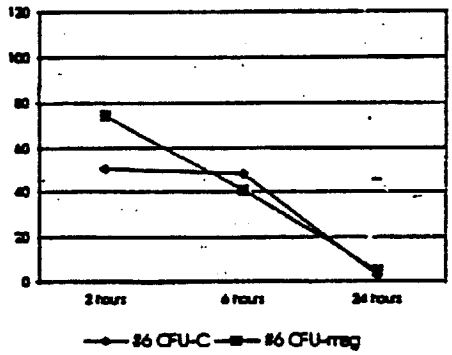












sponsor provided a list of these transcripts. We examined the list and did not come across any obvious relationship which would raise our level of concern regarding the toxicity of CMA-676.

RNA Expression Frequency of Glutathione Metabolism Genes, MDR Genes, and Actin Genes

		mRNA molecules per million				
Genbank ID	Genbank Description	BM# 2	BM# 1	BM# 3	BM# 6	BM# 4
Y00433	Human mRNA for glutathione peroxidase (EC 1.11.1.9.)	202	229	182	164	196
M24485	Homo sapiens (clone pHGST-pi) glutathione S-transferase pi (GSTP1) gene	172	194	160	145	162
U77604	Human microsomal glutathione S-transferase (GST-II) mRNA	49	37	24	55	38
D71973	H.sapiens GPx-4 mRNA for phospholipid hydroperoxide glutathione peroxidase	49	41	48	111	43
U46499	Human microsomal glutathione transferase (GST12) gene	43	61	58	55	50
U90313	Human glutathione-S-transferase homolog mRNA	43	46	50	41	27
D13315	Human mRNA for lactoyl glutathione lyase	39	33	27	42	34
U86529	Human glutathione transferase Zeta 1 (GSTZ1) mRNA	15	21	17	17	13
D00632	Human plasma (extracellular) mRNA for glutathione peroxidase	8	4	8	12	6
U34683	Human glutathione synthetase mRNA	6	6	6	6	6
U67614	Human sinusoidal reduced glutathione transporter-associated protein (HsGshT) mRNA	4	4	4	4	4
J05459	Human glutathione transferase M3 (GSTM3) mRNA	Absent	Absent	Absent	Absent	Absent
L02321	Human glutathione S-transferase (GSTM5) mRNA	Absent	Absent	Absent	Absent	Absent
L38503	Homo sapiens glutathione S-transferase theta 2 (GSTT2) mRNA	Absent	Absent	Absent	Absent	Absent
M16594	Human glutathione S-transferase Ha subunit 2 (GST) mRNA	Absent	Absent	Absent	Absent	Absent
M96233	Human glutathione transferase class mu number 4 (GSTM4) gene	Absent	Absent	Absent	Absent	Absent
U21689	Human glutathione S-transferase-P1c gene	Absent	Absent	Absent	Absent	Absent
X15722	Human mRNA for glutathione reductase (EC 1.6.4.2)	Absent	Absent	Absent	Absent	Absent
X85727_cds2	GSTalpha locus gene (glutathione S-transferase) extracted from H.sapiens GSTalpha gene for glutathione S-tranferase exon 2	Absent	Absent	Absent	Absent	Absent
X68314	H.sapiens mRNA for glutathione peroxidase-GI	Absent	Absent	Absent	Absent	Absent
Z84718_cds1	GSTT2 glutathione transferases 4E-binding protein 1 pseudogene	Absent	Absent	Absent	Absent	Absent
M14758	Human P-glycoprotein (MDR1) mRNA	Absent	Absent	Absent	Absent	Absent
X58723	Human MDR1 (multidrug resistance) gene for P-glycoprotein	Absent	Absent	Absent	Absent	Absent
M10277	Human cytoplasmic beta-actin gene	207	227	171	184	218
X00351	Human mRNA for beta-actin	281	289	227	215	278

RNA levels were analyzed by Affymetrix HuGeneFL arrays. All glutathione and MDR genes represented on the array are shown. If all five bone marrow samples had values considered to be absent by the GeneChip™ software, "Absent" is shown. If at least one sample was considered to be positive by GeneChip™ then the frequency is shown for all samples. Frequency per million was determined by generating a standard curve of hybridization intensity versus known frequency of 11 controls.

PHARMACOKINETICS AND TOXICOKINETICS

GTR-30503 **CMA-676: Biodistribution and Mass Balance of ^{3}H-CMA-676 Following a Single Intravenous Administration in Rats (Study A9463).** Conducted by Wyeth-Ayerst Research, Pearl River, NY.

Methods

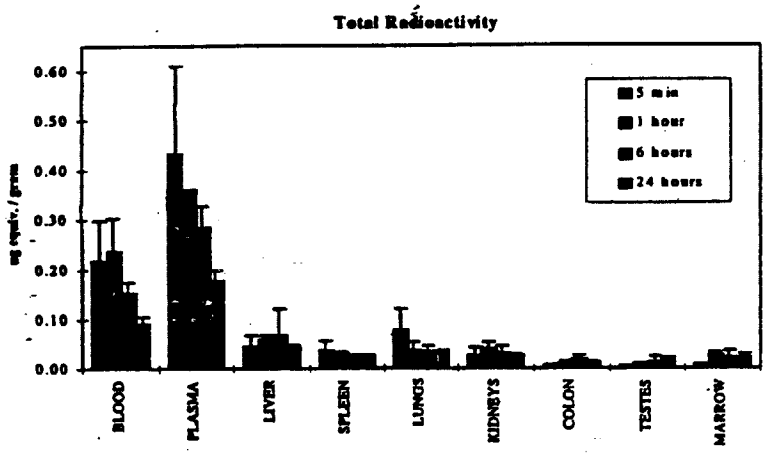
Species and Strain:	Male Sprague Dawley rats
Weight:	~320 g
Drug:	CMA-676
Batch number:	Unlabeled: 9551C-39 ^{3}H-labeled: 9551C-40 Specific activity: 5.7 μCi/μg
Dose administration:	1.3 mg/kg of antibody conjugate (corresponds to 29.5 μg/kg calicheamicin) bolus injection (0.35 ml over 15-20 sec) via the lateral vein
Sample collection:	tissue removal at 0.083, 1, 6, 24, 48, 96, 168, 264, and 336 hrs after dosing; urine and feces were collected from the 240 and 336 hr animals at 24 hr intervals for the duration of the study
Sample analysis:	Tissue samples including urine and feces were homogenized in distilled water when necessary, combusted and counted in a liquid scintillation counter to obtain total radioactivity values. Concentrations of radioactivity are reported as μg equivalents per gram or ml of calicheamicin.
Data analysis:	PK parameters were calculated using model independent methods. A value for C_0 was determined by the method of residuals using the RstripII computer package.

Results

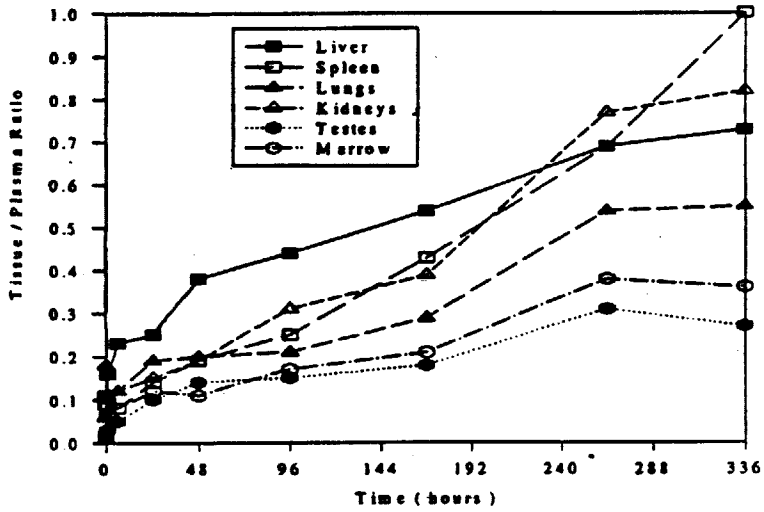
Pharmacokinetic parameters of ^{3}H-CMA-676 (total radioactivity) in selected tissues in rats

Tissue	C_{max} (μg equiv/g or ml)	T_{max} (hr)	$AUC_{0-\infty}$ (μg.equiv.hr/ml)	Cl_T (ml/min/kg)	$T_{1/2}$ (hr)
Blood	0.24	1	10.2	0.05	84
Plasma	0.43	0.083	18.8	0.03	81
Liver	0.07	6	7.90	0.06	130
Spleen	0.04	0.083	8.39	0.06	281
Lung	0.08	0.083	5.15	0.09	153
Kidney	0.04	1	7.76	0.06	246
Colon	0.02	6	2.06	0.24	115
Testes	0.02	24	2.86	0.17	134
Marrow	0.03	1	3.62	0.14	160

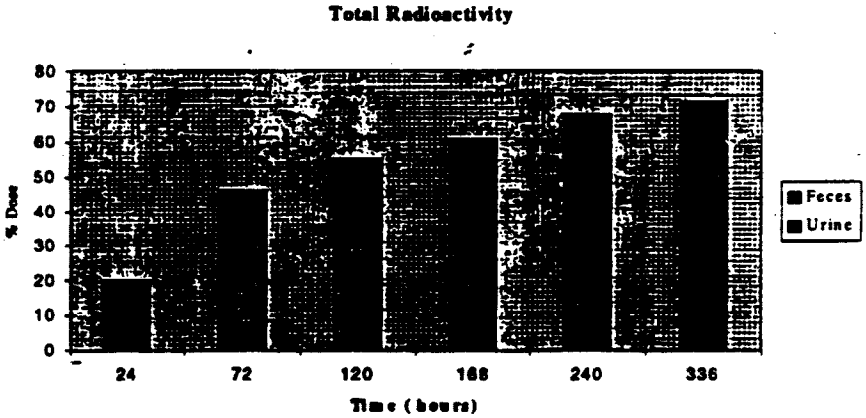
Peak concentrations of radioactivity in tissues were generally observed at the 1 or 6 hr sampling time with lungs, liver, kidney, heart, bone marrow, and adrenals having the highest concentrations of radioactivity from the tissues sampled.



Tissue/plasma ratios were generally < 0.3 for the first 24 hrs, indicating that radioactivity was not rapidly distributed beyond the plasma compartment.



Urine and feces were collected at 24 hr intervals for the duration of the study. Cumulative recovery (% dose) for CMA-676 group was 12.6 and 58.6% in the urine and feces, respectively. Total recovery over the 14-day period was 71.2%.



GTR-34359 **CMA-676: (With Rat Serum Albumin): Intravenous Developmental Toxicity Dose Ranging Study in Gravid Rats: Bioanalytical and Toxicokinetics Report (Protocol 97061).** Conducted by Wyeth-Ayerst Research, Pearl River, NY.
Date of study initiation: 4/7/98

Methods

Species and Strain:	Time-mated female rats (12/group)			
Weight:	~320 g			
Drug:	CMA-676			
Batch number:	Unlabeled:	9551C-39	^{3}H-labeled:	9551C-40
			Specific activity:	5.7 μCi/μg
Dose administration:	0.025, 0.075, and 0.2 mg/kg/day once daily by IV injection via tail vein from gestation days (GD) 6, 10, 14, and 17.			
Sample collection:	Blood (1 ml) was collected pre-dose on GDs 6, 10, 14, and 17 and at 0.083, 2, 6, 12 and 24 hrs after the GD 17 dose.			
Sample analysis:	All samples were assayed for concentrations of hP67.6 antibody while pre-dose samples on GD 6, 10, 14 and samples obtained 24 hrs after			

dosing on GD 17 were also assayed for antibodies to hP67.6 antibody and to the calicheamicin derivative.

Quantitation of hP67.6 antibody in rat plasma:

Plasma concentrations of hP67.6 antibody were determined using a quantitative sandwich ELISA. Quantitation of plasma concentration of hP67.6 antibody included the antibody conjugate, the unconjugated hP67.6 antibody, and the fragments of the antibody conjugate or of the unconjugated hP67.6 antibody which recognize the CD-33 antigen. Immobilized CD33 antigen was coated onto a microtiter plate for binding with hP67.6 antibody present in samples.

Detection of specific antibodies to hP67.6 antibody in rat plasma:

The occurrence of anti-hP67.6 antibodies in the rat was detected using a qualitative sandwich ELISA. The $F(ab')_2$ fragment of hP67.6 antibody was coated onto a microtiter plate. Anti-hP67.6 antibodies present in the samples were bound by the immobilized $F(ab')_2$ fragment of hP67.6 antibody.

Detection of specific antibodies to calicheamicin derivatives in rat plasma:

The occurrence of anti-NAc-gamma calicheamicin DMHAcBut antibodies in the rats was detected using qualitative sandwich ELISA. Biotin-conjugated NAc-gamma calicheamicin DMHAcBut Pentyl amine was added to strepavidin coated plates. Anti-calicheamicin antibodies present in the samples were bound by the immobilized biotin-calicheamicin conjugate.

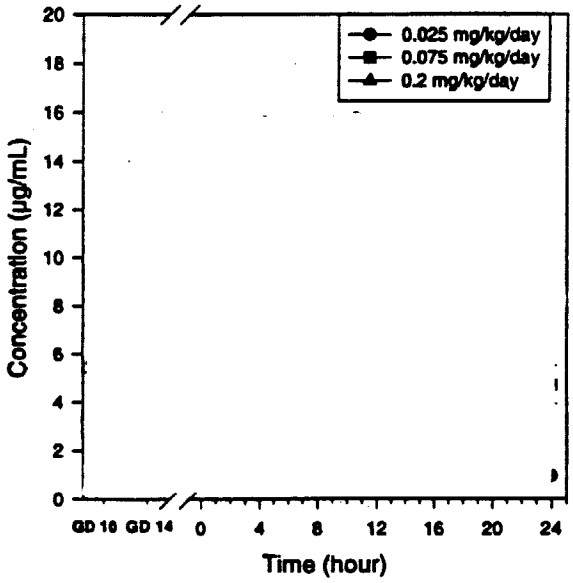
Pharmacokinetic calculations:

Pharmacokinetic parameters were calculated using the pharmacokinetic analysis program WinNonlin, version 1.1 from the group mean concentration vs. time profiles. Data were analyzed using model-independent approach.

Results

Mean concentrations of hP67.6 antibody in gravid rats given a single IV dose of CMA-676

Gestation day	Time (hr)	Mean (± SD) Concentration (µg/ml)		
		0.025 mg/kg/day	0.075 mg/kg/day	0.2 mg/kg/day
6	0	0	0	0
10	0	0.98 ± 0.12	3.58 ± 0.46	7.35 (n = 2)
14	0	1.43 ± 0.10	4.32 ± 0.37	10.4 ± 0.5
17	0	1.20 ± 0.08	4.47 ± 0.27	12.4 ± 0.3
	0.083	1.59 ± 0.14	5.84 ± 1.25	15.4 ± 2.7
	2	1.64 ± 0.05	5.84 ± 0.54	17.5 (n = 2)
	6	1.52 ± 0.17	5.30 ± 0.27	14.3 ± 0.5
	12	1.20 ± 0.02	5.13 ± 0.54	14.0 ± 1.0
	24	0.95 ± 0.07	4.71 ± 0.80	13.5 ± 1.8

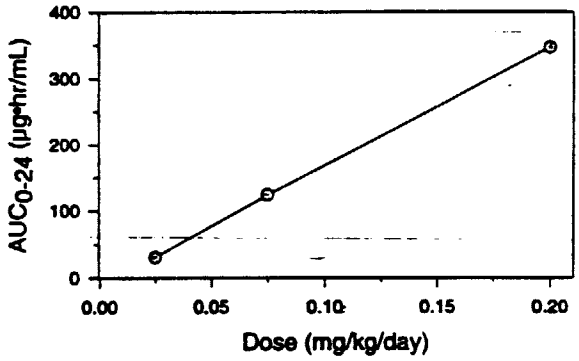


Summary of incidence of formation of antibodies to hP67.6 antibody and calicheamicin

Antibody	Dose (mg/kg/day)	GD 10 (Day 5)	GD 14 (Day 9)	GD 18 (Day 13)
Anti-hP67.6 Antibody				
	0.025	0/3	2/3	4/12
	0.075	0/3	3/3	2/12
	0.200	0/2	3/3	0/9
Anti-calicheamicin				
	0.025	0/3	0/3	0/12
	0.075	0/3	1/3	0/12
	0.200	0/2	0/3	0/9

Pharmacokinetic parameters of hP67.6 antibody in gravid rats given single daily IV CMA-676

Dose (mg/kg/day	$C_{2\ hrs}$ (µg/ml)	$AUC_{0\text{-}24\ hr}$ (µg.hr/ml)	$AUC_{0\text{-}24\ hr}$/Dose	CL_T (ml/min/kg)
0.025	1.64	30.6	1225	0.0136
0.075	5.84	124	1656	0.0101
0.200	17.5	346	1729	0.00964



Conclusion: In gravid rats dosed with CMA-676 once daily between gestation days 6 and 17, exposure to hP67.6 antibody increased with increasing dose. In view of the long terminal half-life values reported in rats and of the relatively short duration of sample collection (up to 24 hr post-dose) in this study, terminal half-life values were not estimated for this study. In addition, accurate estimate of Vd_{ss} could not be obtained due to the same reason. Although some immune response were observed, the titers were relatively low. The presence of antibodies to hP67.6 antibody did not affect the multiple dose pharmacokinetics of total hP67.6 antibody after once daily IV dosing of CMA-676 for 12 days.

GTR-27093 **hP67.6: Pharmacokinetic Comparison in the Monkey of Monoclonal Antibody hP67.6 Derived from Two Different Cell Lines (Protocol 95418).** Conducted by Wyeth-Ayerst Research, Pearl River, NY.

Methods

Species and Strain:	Cynomolgus monkeys (4/group)
Weight:	3.6 – 5.8 kg
Drug:	CMA-676
Batch number:	hP67.6 (old cell line): 25121PC hP67.6 (new cell line): 26372QC
Dose administration:	The actual dose the animals received via the saphenous vein was equivalent to 1.8 mg/kg of hP67.6. The vehicle used for both dosing solutions was 50 mM sodium acetate buffer (pH 7.0) containing 100 nM NaCl.

Sample collection: Blood (2 ml) was collected at 0.083, 0.25, 0.5, 2, 4, 12, 24, 48, 72, 120, 144, 168 and 240 hrs after dosing.

Sample analysis: Plasma concentrations of hP67.6 were determined using an ELISA.

Results The mean (±SD) AUC_{0-240} values for the old and new cell lines were 1946 ± 317 and 3350 ± 636 µg.hr/ml, respectively. These data indicate that exposure (AUC_{0-240}) of hP67.6 was significantly higher (1.7-fold) in monkeys given hP67.6 derived from the new cell line compared to monkeys given hP67.6 derived from the old cell lines. However, in a recently completed study (not reviewed) the PK parameters of hP67.6 were similar in monkeys given CMA-676 manufactured from old or new cell lines. Therefore, the significance of the results of this study is uncertain.

GTR-35324 **CMA-676: Characterization of *In vitro* Metabolism of NAc-Gamma-Calicheamicin DMH (CL-184538) and NAc-Gamma-Calicheamicin DMH AcBUT Acid (CL-191305) in Human Liver Microsomes and Cytosol.** Conducted by Wyeth-Ayerst Research, Pearl River, NY.

Key findings: NAc-γ-calicheamicin DMH AcBut acid is rapidly hydrolyzed to NAc-γ-calicheamicin DMH *in vitro*. Metabolites produced from these two compounds in human liver microsomes were demethylated and oxygenated calicheamicin products. Conversely, the epsilon derivatives were produced in cytosol.

Method

Incubation of NAc-γ-calicheamicin DMH and NAc-γ-calicheamicin DMH AcBut acid with Human Liver Microsomes

NAc-γ-calicheamicin DMH (7 µM) or NAc-γ-calicheamicin DMH AcBut acid (6 µM) was incubated with 2 mg of human liver microsomal protein with or without 60 µl NADPH generating system (1.3 mM NADP, 3.5 mM glucose-6-phosphate, 0.4 units/ml glucose-6-phosphate dehydrogenase), 10 mM $MgCl_2$ and 2 mM EDTA in 0.1 M potassium phosphate buffer (pH 7.4) for 2 hr at 37°C. Control sample without NADPH generating system or microsomes or incubation (time 0), were also run. Higher than therapeutic concentrations were used in the incubations to produce adequate amounts of metabolites for characterization by [redacted] All these samples were analyzed by [redacted] and selected samples were analyzed by [redacted]

Incubation of NAc-γ-calicheamicin DMH and NAc-γ-calicheamicin DMH AcBut acid with Human Liver Cytosol

NAc-γ-calicheamicin DMH (7 µM) or NAc-γ-calicheamicin DMH AcBut acid (6 µM) was incubated in cytosol under similar conditions as described for the microsomes except that 5 mg of cytosolic protein and 90 µl NADPH generating system (2 mM NADP, 5.3 mM glucose-6-phosphate, 0.6 units/ml glucose-6-phosphate dehydrogenase) were used, and the incubation time was 3 hr. For additional MS characterizations, higher than therapeutic concentrations were used and incubated for longer period to produce adequate amounts of metabolites. All these samples were analyzed by [redacted] and selected samples were analyzed by [redacted]

Results

Metabolite profiles of NAc-γ-calicheamicin DMH in Human Liver Microsomes and Cytosol [redacted] chromatograms were submitted but not shown in this review)

No metabolites were found in microsomes in the absence of the NADPH. Only a small peak (M3) was detected in the time 0 sample. In contrast, three metabolites (M1, M2, and M10) were found after incubation for 2 hr.

Two metabolites (M3 and M4) were detected in cytosol in samples without incubations (time 0). Five metabolites (M4, M6, M7, M8, and M9) were found after incubation without NADPH; the retention time of M6 was similar to the NAc-epsilon calicheamicin standard. Metabolites M5, M6, M7, and M8 were detected in the sample incubated with NADPH. Formation of M5 is NADPH dependent. Also, in the presence of NADPH, the M9 peak was absent and M7 decreased significantly.

Metabolite profiles of NAc-γ-calicheamicin DMH AcBut acid in Human Liver Microsomes and Cytosol (chromatograms were submitted but not shown in this review)

No metabolites were found in microsomes in the absence of the NADPH. NAc-γ-calicheamicin DMH AcBut acid was rapidly and extensively converted to NAc-γ-calicheamicin DMH. The profiles observed were similar to those after incubation of NAc-γ-calicheamicin DMH with microsomes.

NAc-γ-calicheamicin DMH AcBut acid was converted to NAc-γ-calicheamicin DMH in cytosol but not as extensively as was observed in microsomes. A small peak of retention time similar to M3 was present at time 0. The profiles generated after incubation in human liver cytosol with and without NADPH were similar to the profiles observed for NAc-γ-calicheamicin DMH.

Mass Spectrometric Analysis

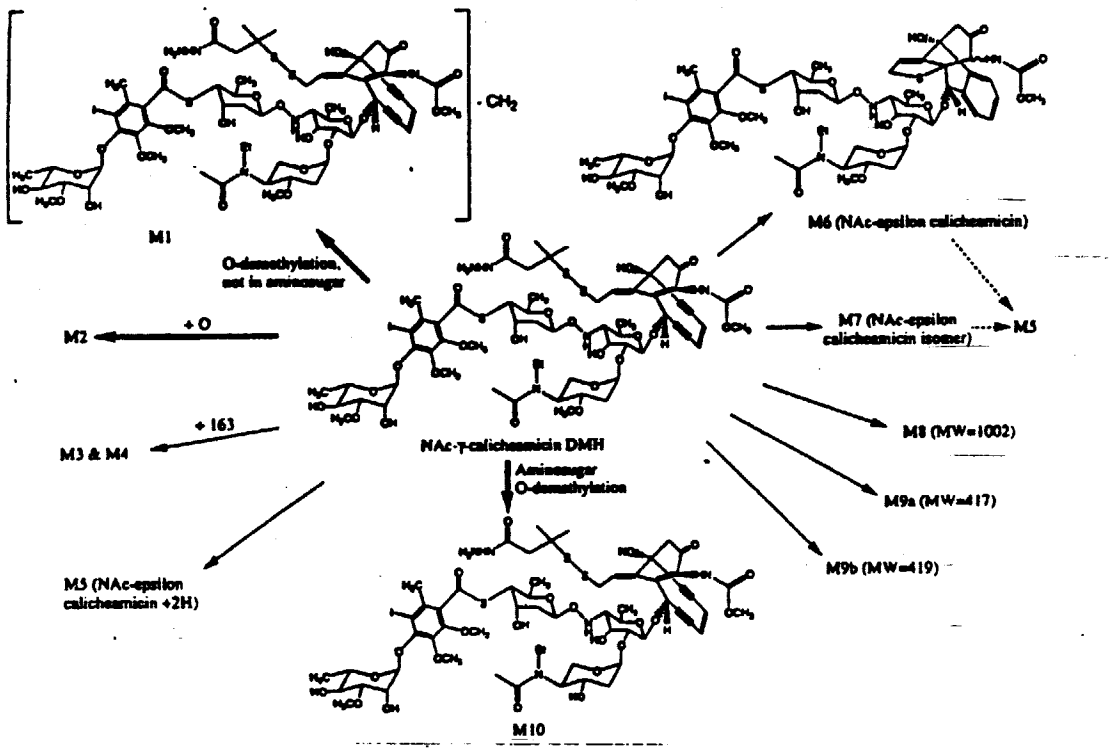
The analysis of metabolites of NAc-γ-calicheamicin DMH and NAc-γ-calicheamicin DMH AcBut acid in human liver microsomes and cytosol are summarized in following tables. Metabolites peaks M11a, M11b, M12, M13, M15a, and M15b were not conspicuous from HPLC/UV analysis.

Summary of analysis of samples after incubation of NAc-γ-calicheamicin DMH AcBut in human liver microsomes (HLM) and cytosol (HLC)

Metabolite	Enzyme system	MS Retention time	Molecular Wt.	Comments
M1	HLM	30:09	1463	O-demethylation
M2	HLM	38:00	1493	Hydroxylation
M3	HLC	34:45	1640	Contains amino sugar
M4	HLC	36:00	1640	Contains amino sugar
M5	HLC	28:15	1335	NAc-epsilon calicheamicin + 2H
M6	HLC	39:31	1333	NAc-epsilon calicheamicin
M7	HLC	47:06	1333	NAc-epsilon calicheamicin isomer
M8	HLC	24:10	1002	Contains amino sugar
M9a	HLC	14:00	417	Inconclusive
M9b	HLC	13:49	419	Inconclusive
M10	HLM	27:47	1463	O-demethylation (amino sugar)
DMH*	HLM	43:06	1477	DMH
	HLC	43:15		

*DMH = NAc-γ-calicheamicin

PROPOSED METABOLIC PATHWAY OF NAc-γ-CALICHEAMICIN DMH IN HUMAN LIVER MICROSOMES (➨) AND CYTOSOL (→)



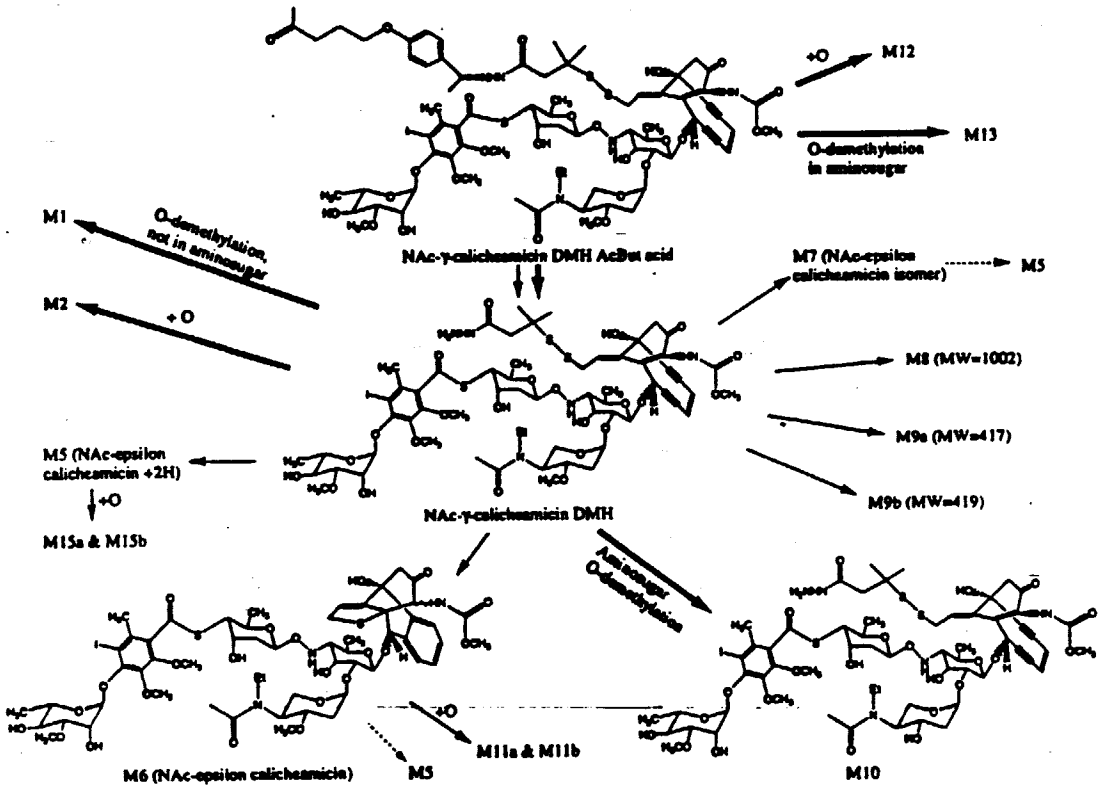
Summary of analysis of samples after incubation of NAc-γ-calicheamicin DMH in human liver microsomes (HLM) and cytosol (HLC)

Metabolite	Enzyme system	MS Retention time	Molecular Wt.	Comments
M1	HLM	29:51	1463	O-demethylation
M2	HLM	37:15	1493	Hydroxylation
M5	HLC	28:00	1335	NAc-epsilon calicheamicin + 2H
M6	HLC	39:01	1333	NAc-epsilon calicheamicin
M7	HLC	46:35	1333	NAc-epsilon calicheamicin isomer
M8	HLC	23:37	1002	Contains amino sugar
M9a	HLC	14:00	417	Inconclusive
M9b	HLC	13:49	419	Inconclusive
M10	HLM	27:08	1463	O-demethylation (amino sugar)
M11a	HLC	26:04	1349	NAc-epsilon calicheamicin + O
M11b	HLC	27:26	1349	NAc-epsilon calicheamicin + O
M12	HLM	40:48	1697	AcBut acid + O
M13	HLM	31:26	1667	AcBut acid O-demethylation (amino sugar)
DMH*	HLM	42:30	1477	DMH
	HLC	42:28		
M15a	HLC	19:03		NAc-epsilon calicheamicin + 2H + O
M15b	HLC	20:51		NAc-epsilon calicheamicin + 2H + O
AcBut acid **	HLM	43:24		AcBut acid
	HLC	43:35		

*DMH = NAc-γ-calicheamicin DMH

**AcBut acid = NAc-γ-calicheamicin DMH AcBut acid

PROPOSED METABOLIC PATHWAY OF NAc-γ-CALICHEAMICIN DMH AcBUT ACID IN HUMAN LIVER MICROSOMES (➡) AND CYTOSOL (⇢)



GTR-35325 **CMA-676: Characterization of *in vitro* Metabolism of NAc-Gamma-Calicheamicin DMH (CL-184538) in HL-60 Promyelocytic Leukemia Cells.** Conducted by Wyeth-Ayerst Research, Pearl River, NY.

Key findings: Five metabolites (M6, M7, M8, M14 and M16) were produced, when NAc-γ-calicheamicin DMH was incubated with HL-60 leukemia cells. The detection of NAc-epsilon calicheamicin (M6) and another isomer of NAc-epsilon calicheamicin (M7) lends support to the hypothesis that the reactive diradical calicheamicin epsilon species may be formed via a glutathione-dependent reduction of the disulfide bond of NAc-γ-calicheamicin DMH within cells.

Method

NAc-γ-calicheamicin DMH was incubated with HL-60 promyelocytic leukemia cells at 3 and 10 μM using 10 and 20 million cells per incubation and reactions were terminated at various times, up to 24 hr post-treatment. The samples were extracted with ethyl acetate and analyzed by

Results

Metabolite profiles

Two metabolites (M6 and M16) were identified after incubation of NAc-γ-calicheamicin DMH at 3 μM with 10 million cells for 4 hr. No metabolite peaks were detected in the 0 and 1 hr samples. M6 was identified as NAc-epsilon calicheamicin. The data on the LC/MS analysis of M16 was inconclusive. Another metabolite (M7) was produced after incubation of 10 μM NAc-γ-calicheamicin DMH with 20 million cells for 24 hr. M7 was identified

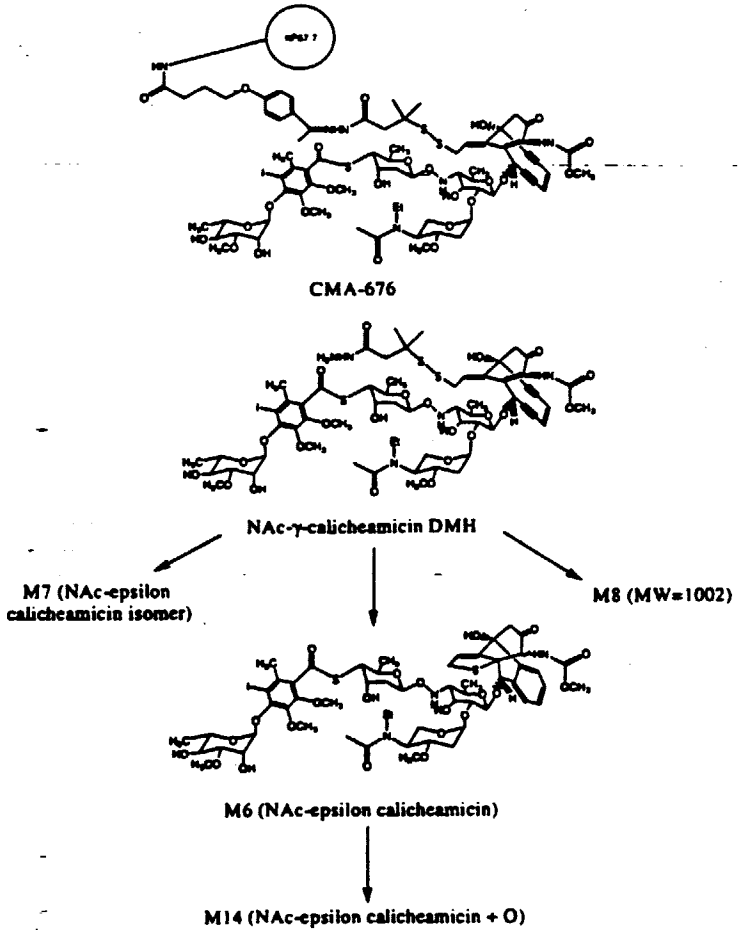
as an isomer of NAc-epsilon calicheamicin based on the same molecular weight and product ion mass spectrum as NAc-epsilon calicheamicin. The formation of M6 and M7 were time dependent and it was slower for M7.

Mass spectrometric analysis of metabolites
Two additional metabolites (M14 and M8) which were not conspicuous in the analysis were also detected. Results are summarized in the table below.

Summary of the LC/MS analysis of metabolites of NAc-γ-calicheamicin DMH in HL-60 leukemia cells

Peak	Molecular wt.	Retention Time (min)	Possible identity
NAc-γ-calicheamicin DMH	1477	44.67	NAc-γ-calicheamicin DMH
M6	1333	40.95	NAc-epsilon calicheamicin
M7	1333	48.99	Isomer of NAc-epsilon calicheamicin
M8	1002	25.18	Inconclusive
M14	1349	24.08	NAc-epsilon calicheamicin + O
M16		34.85	Inconclusive

STRUCTURE OF CMA-676 (CL-555201) AND THE PROPOSED METABOLIC PATHWAY OF NAc-γ-CALICHEAMICIN DMH (CL-184538) IN HL-60 PROMYELOCYTIC LEUKEMIA CELLS



REPRODUCTIVE TOXICITY

GTR-33248 **CMA-676 (With Rat Serum Albumin): Intravenous Developmental Toxicity Dose Ranging Study in Gravid Rats.** Conducted by Wyeth-Ayerst Research, Drug Safety, Chazy, NY, in compliance with GLP regulations. Quality assurance statement was also included.
Date of study initiation: 4/7/98

Key findings: Daily maternal treatment of rats with CMA-676 during GD 6 – 17 produced dose-related decreases in fetal weights at the doses of 0.025 and 0.075 mg/kg, dose-related increases in embryo/fetal mortality at 0.075 and 0.2 mg/kg, and fetal digital malformations at the dose of 0.075 mg/kg. At the dose of 0.2 mg/kg, the resorption of all implantations precluded fetal evaluations.

Method

Species:	Time-mated female CD VAF rats	
	Developmental toxicity	8/group
	Toxicokinetics	12/group
	Diluent control:	
	1% rat serum albumin & 1.55% sucrose in PBS	3/group
	0.91% dextran-40 in PBS	3/group
	CMA-676 formulation with less dextran-40 (0.075 & 0.150 mg/kg/day)	5/group
Age & weight:	9 to 12 weeks; 194 – 282 g	
Test article:	CMA-676, lyophilized monoclonal antibody conjugate, 5 mg-vial containing CMA-676, sodium phosphate dibasic, sodium phosphate monobasic, sucrose, dextran-40, and sodium chloride	
Lot number:	040497-PSG2024-13	
Control article (diluent):	Phosphate buffered saline with 1.0% rat serum albumin and 1.55% sucrose, and 0.91% dextran-40.	
Dosages:	0, 0.025, 0.075, and 0.2 mg/kg/day, GD 6-17	

Observations

Clinical signs:	Beginning on GD 6, once daily for clinical observations and twice daily for mortality
Body weight:	Daily on GD 6 through 18 and on GD 21
Food consumption:	GD 6 through 9, 10 through 13, 14 through 17, and 18 through 20
Gross pathology:	On GD 21 gravid uterus and ovaries were excised, weighed and following observation were made: *Hysterotomy findings:* corpora lutea counted in each ovary, implantations evaluated as late or early resorptions, dead or live fetuses *Offspring observations:* fetuses examined for palatal closure, gross external abnormalities, and gender *Gross appearance of placenta*

Results

Clinical signs:	No drug related mortality Thin appearance in 1 HD animal in association with body wt. loss, low food consumption, and full litter resorption ***Effect related to the administration of the vehicle*** All animals treated with dextran-40 (0.91%) in PBS as vehicle had swelling in the front and hind feet and nose. Tissue swelling did not occur in the rats given formulation diluent without dextran-40. In addition, CMA-676 formulations containing lower concentrations of dextran-40 did not produce tissue swelling.

	0.025 mg/kg	0.075 mg/kg	0.2 mg/kg
Maternal body wt. gain (GD 6 – 17)	↓ 16 %	↓ 60 %	*
Maternal body wt. gain (GD 18 – 20)	↓ 12 %	↓ 74 %	*
Gravid uterine wt.	↓ 11 %	↓ 79 %	↓ 98 %
Maternal body wt. (GD 21)	↓ 4 %	↓ 22 %	↓ 36 %
Maternal food consumption values	↓ 4 %	↓ 17 %	↓ 28 %
Maternal food consumption values (during post-dosing period)		↓ 9 %	↓ 41 %

*No body wt. gain. Loss of 5.4 g during GD 6 – 17 and loss of 3.9 g during GD 18 – 20.

Hysterotomy findings: The mean number of corpora lutea and implantations per dam were comparable among all groups

	Control	0.025 mg/kg	0.075 mg/kg	0.20 mg/kg
Embryo/fetal mortality				
Early resorptions per litter	1.13	0.75	10.13	13.38
Late resorptions per litter	0	0	0.13	
Average # of live fetuses per litter	10.88	11.13	2.88	0

Examination of Offspring:

Fetal sex distribution The proportions of male and female fetuses were not affected in LD & MD animals. This parameter could not be evaluated in the HD group.

Fetal body wt. average wts. were decreased 9% and 41% in the LD & MD animals, respectively.

Fetal gross external examination 3 of 5 litters with live fetuses or 5 of 23 fetuses in the MD group had digital malformations (ectrodactyly, polysyndactyly, and brachydactyly) in one or both hind feet. There were no fetal gross external anomalies in the LD group. This parameter could not be evaluated in the HD group.

Gross appearance of placenta: Unremarkable in MD & HD group. This parameter could not be evaluated in the HD group.

GTR-33829 **CMA-676: Intravenous Developmental Toxicity Study in Gravid Rats.** Conducted by Wyeth-Ayerst Research, Drug Safety, Chazy, NY, in compliance with GLP regulations. Quality assurance statement was also included.
Date of study initiation: 5/25/98

Key findings: Daily maternal treatment of rats with CMA-676 during GD 6 – 17 at 0.060 mg/kg produced increased embryo-fetal mortality, decreased numbers of live fetuses per litter, and low fetal incidences of gross external, visceral, and skeletal anomalies. At the dose of 0.025 mg/kg, fetal skeletal anomalies were present in a single fetus. In the 0.010 mg/kg group, only a slight decrease in skeletal ossification was observed. Affects on fetal weight, ossification, and wavy ribs became apparent at 0.025 mg/kg/day.

Method

Species:	Time-mated female CD VAF rats (25/group)
Age & weight:	9 to 11 weeks; 208 – 256 g
Test article:	CMA-676, lyophilized monoclonal antibody conjugate, 5 mg-vial containing CMA-676, sodium phosphate dibasic, sodium phosphate monobasic, sucrose, dextran-40, and sodium chloride
Lot number:	040497-PSG2024-13
Control article (diluent):	Phosphate buffered saline with 1.0% rat serum albumin and 1.55% sucrose.
Dosages:	0, 0.010, 0.025, 0.060 mg/kg/day, GD 6-17

Observations

Clinical signs:	Beginning on GD 6, once daily for clinical observations and twice daily for mortality
Body weight:	Daily on GD 6 through 18 and on GD 21
Food consumption:	GD 6 through 9, 10 through 13, 14 through 17, and 18 through 20
Gross pathology:	On GD 21 gravid uterus and ovaries were excised, weighed and following observation were made: *Hysterotomy findings:* corpora lutea, litter size, embryo/fetal mortality *Offspring observations:* gender, weight, gross external and palatal anomalies, and visceral or skeletal anomalies and *Gross appearance of placenta*
Statistical methods:	Body wt., body wt.gains, gravid uterine wt., hysterotomy parameters, fetal sex, fetal examination findings were analyzed for trend using a Jonckheere's test, and for a difference among groups using a nonparametric one way analysis of variance. Analysis of hysterotomy findings were done with respect to number per litter and proportion per litter. Analysis of fetal examination findings were done with respect to proportion of affected fetuses per litter and proportion of litters with at least one affected fetus. Food consumption, and fetal wt. were analyzed for trend using Jonckheere's test and a nonparametric covariance trend test, and a nonparametric one way analysis of variance.

Results

Clinical signs: All females survived to the scheduled euthanasia on GD 21
Single-day occurrence of red pigment around genitalia

	0.010 mg/kg	0.025 mg/kg	0.060 mg/kg
Maternal body wt. gain (GD 6 – 17)	↓ 7 %	↓ 15 %	↓ 43 %
Maternal body wt. gain (GD 18 – 20)	↓ 8 %	↓ 19 %	↓ 56 %
Gravid uterine wt.	↓ 7 %	↓ 17 %	↓ 59 %
Maternal adjusted pregnancy wt. gain (GD 6 – 20)	↓ 7 %	↓ 18 %	↓ 23 %
Maternal food consumption values	↓ 4 %	↓ 4 %	↓ 11 %
Maternal food consumption values (during post-dosing period)		↓ 5 %	↓ 8 %

Hysterotomy findings:	Control	0.010 mg/kg	0.025 mg/kg	0.060 mg/kg
Embryo/fetal mortality				
Early resorptions per litter	0.79	0.42	0.50	4.17
Early resorptions proportion/litter	0.06	0.03	0.05	0.35
Late resorptions per litter	0	0	0	0.46
Late resorptions proportion/litter	0	0	0	0.04
Average # of live fetuses per litter	12	11.75	11.42	7.46
Live fetuses proportion/litter	0.94	0.97	0.95	0.61

No drug-related effect on total implantations, preimplantation loss, and corpora lutea.

Examination of Offspring:	Control	0.010 mg/kg	0.025 mg/kg	0.060 mg/kg
↓ Absolute wt. of fetuses (♂/♀)		3% / 4%	9% / 10%	39% / 40%
Fetal sex distribution (mean proportion of males)	0.53	0.48	0.50	0.51
Fetal gross external examination				
Brachydactyly – Hind				
Proportion/litter	0	0	0	0.06
Proportion of litters	0	0	0	0.17
Ectrodactyly – Hind				
Proportion/litter	0	0	0	0.05
Proportion of litters	0	0	0	0.08
Short tail				
Proportion/litter	0	0	0	0.01
Proportion of litters	0	0	0	0.04
Hind paw digit malformations				
Proportion/litter	0	0	0	0.06
Proportion of litters	0	0	0	0.17
Fetal visceral examination				
Absence of Aortic arch				
Proportion/litter	0	0	0	0.01
Proportion of litters	0	0	0	0.05
Hemorrhage in brain				
Proportion/litter	0	0	0	0.05
Proportion of litters	0	0	0	0.05
Fetal skeletal examination				
Humerus – Short/Thick				
Proportion/litter	0	0	0	0.02

Proportion of litters	0	0	0	0.09
Radius – Misshapen				
Proportion/litter	0	0	0	0.02
Proportion of litters	0	0	0	0.09
Scapula – Misshapen				
Proportion/litter	0	0	0	0.02
Proportion of litters	0	0	0	0.09
Ulna – Misshapen				
Proportion/litter	0	0	0	0.02
Proportion of litters	0	0	0	0.09
Ulna – Short/Thick				
Proportion/litter	0	0	0	0.01
Proportion of litters	0	0	0	0.04
Ribs – Decreased number				
Proportion/litter	0	0	0.01	0
Proportion of litters	0	0	0.04	0
Fused – Sternebrae				
Proportion/litter	0	0	0	0.01
Proportion of litters	0	0	0	0.04
Hemivertebrae/missing vertebral element				
Proportion/litter	0	0	0.01	0.04
Proportion of litters	0	0	0.01	0.04
Metacarpal – Reduced number Ossified				
Proportion/litter	0.01	0	0	0.07
Proportion of litters	0.08	0	0	0.13
Phalanges – Reduced number Ossified-Front				
Proportion/litter	0.21	0.20	0.15	0.53
Proportion of litters	0.63	0.58	0.50	0.83
Wavy ribs				
Proportion/litter	0.02	0.01	0.08	0.37
Proportion of litters	0.04	0.04	0.33	0.61
Basiphenoid – Enlarged Canal/Cleft				
Proportion/litter	0.02	0.01	0.01	0.15
Proportion of litters	0.08	0.08	0.08	0.30
Interparietal – Reduced Ossification				
Proportion/litter	0.00	0.03	0.05	0.04
Proportion of litters	0.00	0.13	0.21	0.17
Parietal – Reduced Ossification				
Proportion/litter	0.03	0.04	0.08	0.09
Proportion of litters	0.17	0.17	0.33	0.17
Sternebrae – Reduced number Ossified				
Proportion/litter	0.05	0.02	0.06	0.48
Proportion of litters	0.25	0.13	0.25	0.83
Caudal vertebrae – Unossified				
Proportion/litter	0.01	0.01	0.02	0.26
Proportion of litters	0.08	0.04	0.08	0.48
Vertebral centra – Reduced number Ossified				
Proportion/litter	0.16	0.17	0.27	0.98
Proportion of litters	0.46	0.50	0.83	1.00
Decreased ossification of skeleton				
Proportion/litter	0.36	0.37	0.49	0.98
Proportion of litters	0.71	0.88	0.96	1.00

Placental morphology: Paleness of two placentae, one each in MD and HD groups, were not considered drug-related (no reason provided)

Overall Reproductive Toxicity Summary

Daily treatment of pregnant rats with 0.01 to 0.06 mg/kg/day CMA-676 during organogenesis caused dose related decreases in fetal weight in association with dose-related decreases in fetal skeletal ossification. These findings were clearly evident beginning at 0.025 mg/kg/day. Doses of 0.060 mg/kg/day produced increased embryo-fetal mortality (increased numbers of early and late resorptions and decreased numbers of live fetuses per litter). Five of 179 fetuses (3% overall incidence) from 4 of 24 litters had digital malformations (ectrodactyly; brachydactyly) in one or both hind feet. Similar digital malformations occurred in 22% of examined fetuses at a higher dosage of 0.075 mg/kg in the dose-range finding study. A single fetus in the 0.060 mg/kg group had a short tail and no other gross external or skeletal malformation. Unlike the digital malformations, short tail was not observed in the dose-range finding study at the higher dosage of 0.075 mg/kg. Other visceral, and skeletal alterations at the dose of 0.060 mg/kg included absence of the aortic arch, wavy ribs, anomalies of the long bones in the forelimb(s) (short/thick humerus, misshapen radius, misshapen ulna, and short/thick ulna), misshapen scapula, absence of vertebral centrum, and fused sternebrae.

Reproductive risk integration for CMA-676

The only studies conducted were ICH stage C-D studies with CMA-676 in time-mated female CD VAF rats. Based on the unequivocally positive results obtained from a pilot study, we agreed with the sponsor that one species would be sufficient to support an NDA for CMA-676 in a leukemia indication. Since peri and post-natal studies were not conducted, no information is available on the effect of CMA-676 on parturition, lactation, functional development, locomotion or learning ability.

Factors for Assessment	Fertility	Developmental Mortality	Dysmorphogenesis	Alteration to Growth
Signal Strength I	**+1**	**+1**	**+1**	**+1**
Cross-species Concordance	↑	-	-	-
Multiplicity of effects	-	↑	↑	↑
Adverse Effects as function of time	-	-	-	↑
Signal Strength II	**0**	**+1**	**0**	**+1**
Maternal Toxicity	-	-	-	↑
Dose-Response	↑	↑	-	↑
Rare Events	-	-	-	-
Pharmacodynamics	**+1**	**+1**	**+1**	**+1**
Therapeutic Index	↑	↑	↑	↑
Comparison of Biomarker Benchmarks	-	-	-	-
Similarity Pharmacologic & Toxic mechanism	↑	↑	↑	↑
Concordance between Test Species & Humans	**+1**	**+1**	**+1**	**+1**
Metabolism	-	-	-	-
General Toxicity Profiles	↑	↑	↑	↑
Biomarker Profiles	-	-	-	-
Relative Exposures	**+1**	**+1**	**+1**	**+1**
Class Alerts	**0**	**0**	**0**	**0**
Total	**+4**	**+5**	**+4**	**+5**

Consideration of the individual factors in the draft Reproductive and Developmental Toxicity Integration Tool indicates that there is significant concern for adverse effects of CMA-676 on human fertility, fetal survival, morphogenesis, and fetal growth. Concern is increased for all positive signals after considering the pharmacodynamics, cross species concordance, and relative exposure Factors in the tool. There is no prior human experience with calicheamicins so there are no adjustments for the Class Alert Factor. Consideration of adjustments to overall concern based on Signal Strength factors are discussed with the individual positive endpoints below.

Factors applying equally to all positive endpoints

Pharmacodynamics. Several sets of data were considered for adjusting concern based on the TI contributory element. *a)* Three doses of 0.45 mg/m^2 CMA-676 was effective at curing athymic mice of HL-60 cell tumors. Alterations to fetal growth, survival, and morphogenesis were seen at 0.36 mg protein/m^2 in rats. The $TI_{10/90}$ ratio for these data is 0.8. *b)* The total calicheamicin AUC in humans at the recommended dose was 2.5 ± 1.8 μg•hr/ml whereas the AUC for total calicheamicin derivatives in rats at 8.4 mg protein/m^2 was 9.2 μg-eq•hr/ml. Assuming linear pharmacokinetic in rats and no differences in pharmacokinetics between pregnant and non-pregnant rats, the AUC at the developmentally toxic dose of 0.36 mg protein/m^2 is estimated to be ~0.4 μg-eq•hr/ml. The $TI_{10/90}$ ratio for these AUC data is 0.16. Note, however, that total calicheamicin derivatives includes antibody bound, cleaved, and metabolized calicheamicin residues. The comparative profile of these entities between rats and humans and their individual activities is not known. This $TI_{10/90}$ ratio should be considered a crude estimate. *c)* A dose of 7.2 mg/m^2/week caused testicular toxicity in rats that could reasonably be expected to affect fertility. The $TI_{10/90}$ ratio for fertility relative to the efficacious dose in mice (~ED_{90}) is 16; whereas the $TI_{10/90}$ ratio relative to the recommended human dose is 0.8. Concern is enhanced for this contributory element because the $TI_{10/90}$ was <5 for these comparisons (for fertility effects, the ratio relative to humans was deemed more relevant than relative to mice).

Calicheamicin is presumed to cause death to transformed cells by inducing double-stranded breaks in DNA. Such breaks are difficult to repair and are particularly lethal to rapidly dividing cells, including both malignant and normal cells. Toxicity to germ cells or developing conceptuses is thus considered a direct extension of the mechanism of action of CM-676 and concern is enhanced for the "similarity of pharmacologic and toxicologic mechanism" element. No biomarker benchmark data were available to assess this contributory element. The overall concern is increased +1 for the Pharmacodynamics factor.

Concordance between Test Species & Humans. Metabolism studies were conducted only with human liver microsomes and cytosol and not in a preclinical species. The concordance of metabolism and distribution profiles could thus not be assessed. The general toxicity profile was considered concordant based on the liver toxicity observed in humans, rats, and monkeys. No data was available to assess the concordance of biomarker profiles. The overall concern was increased +1 for the Concordance factor.

Relative exposures. Increased embryo-fetal mortality (increased numbers of resorptions and decreased numbers of live fetuses per litter) and gross external, visceral, and skeletal alterations were observed at a dose approximately 0.04 times the recommended human dose on a mg/m^2 basis. Growth alterations were noted at a dose (0.025 mg/kg/day) approximately 0.017 times the recommended human dose on a mg/m^2 basis. In addition, CMA-676 caused atrophy of the testes in rats at the dose of 1.2 mg/kg/week (approximately 0.8 times the human dose on a mg/m^2 basis). Thus, there is an increase in the level of concern for all the endpoints listed in the table because these effects were observed in the animals at doses lower than the recommended human dose.

Class Alert. Calicheamicin is the prototype member of the enediyne class of antitumor antibiotics. There is no evidence in the literature which would suggest that any compound in this class is involved in causing adverse reproductive effects in humans. Due to the absence of human reproduction data, the level of concern for all the endpoints due to this factor was unchanged.

Other factors and contributory elements specific for each endpoint are discussed below:

Overall Assessment of Concern for Individual Positive Endpoints

Fertility:

Fertility studies were not conducted in animals. However, when given weekly for 6 doses to rats, CMA-676 caused slight to marked atrophy of the seminiferous tubules, slight oligospermia, slight desquamated cells in the epididymis, and slight hyperplasia of the interstitial cells. These findings did not resolve in recovery. Decreased testicular weights were noted in monkeys administered 6 weekly doses of CMA-676. Histopathology changes, however, were not observed in male monkey reproductive organs (these tissues were examined, see histopathology table in IND review). Concern was thus enhanced based on the "cross-species concordance" element for testicular

toxicity. No other effects were noted within the Reproductive Toxicity category because studies were not conducted. Concern was therefore unchanged for the "multiplicity of effects" element. Data was not available other than for terminal sacrifice in the toxicology studies, so the "adverse effects as a function of time" element did not affect concern. Overall concern for fertility was increased +1 for the Signal Strength I factor based on the "cross species concordance" element.

Doses causing testicular toxicity also caused a variety of other serious toxicities. In the absence of evidence that the testicular effects were secondary to paternal toxicity; the testicular toxicity can reasonably be attributed to direct effects of CMA-676. Concern was therefore unchanged for the "paternal toxicity" element. Effects were observed at both MD (↓weight only) and HD in rats, but only HD in monkeys. Concern was enhanced based on the "dose response" element. Gonadal toxicity is not a rare event for cytotoxic anticancer drug, so concern was unchanged for this element. Overall concern for fertility was left unchanged for the Signal Strength II factor since concern was only enhanced by a dose response relationship for indirect indicators of fertility in one species.

Developmental mortality:

CMA-676 produced a dose-dependent increase in embryo-fetal mortality (increased numbers of resorptions and decreased numbers of live fetuses per litter). For the Signal Strength I factor, cross-species concordance could not be assessed because a study was conducted in only one species. In addition to mortality, structural alterations and growth alterations were also observed. Concern is thus enhanced for the "multiplicity of effects" contributory element. Since resorptions occurred at both stages (early and late), the level of concern was also enhanced due to the "adverse effects as a function of time" contributory element. Overall concern for developmental mortality was increased +1 for the Signal Strength I factor.

Embryomortality was seen at doses where maternal toxicity was manifested as a significant decrease in weight (~50% ↓weight gain). It is unknown, however, whether embryomortality was directly caused by maternal toxicity and concern was left unchanged. A clear dose response relationship was noted in conjunction with the range finding study which enhanced concern. Mortality is not a rare event and concern was not adjusted for this element. Overall concern was increased +1 based on the Signal Strength II factor.

Dysmorphogenesis:

Gross external, visceral, and skeletal alterations at this dose included digital malformations (ectrodactyly, brachydactyly) in one or both hind feet, absence of the aortic arch, wavy ribs, anomalies of the long bones in the forelimb(s) (short/thick humerus, misshapen radius, misshapen ulna, and short/thick ulna), misshapen scapula, absence of vertebral centrum, and fused sternebrae. For Signal Strength I, concern was not adjusted for "cross-species concordance" (only one study) nor "adverse effects as a function of time" (only one sacrifice time). In addition to structural alterations, mortality and growth alterations were also observed. Concern is thus enhanced for the "multiplicity of effects" contributory element. Overall concern for dysmorphogenesis was increased +1 for the Signal Strength I factor.

Although maternal toxicity was evident at the dose causing skeletal and visceral malformations it is unknown whether the structural effects were due solely to secondary effects of maternal toxicity. Based on the mechanism of action, it is reasonable to attribute these findings to direct effects of CMA-676. Concern was unchanged for "maternal toxicity" considerations. The dose-response relationship for these malformations was not observed in the developmental toxicity study because these malformations were only seen at the highest dose of 0.060 mg/kg. In the range-finding study, all the fetuses were dead at the highest dose and malformations were thus only seen at 0.075 mg/kg/day. Concern was not adjusted for the "dose-response" element. None of the malformations were considered rare. Overall concern for dysmorphogenesis was unchanged by the Signal Strength II factor.

Alterations to growth:

Daily treatment of pregnant rats with 0.01 to 0.06 mg/kg/day CMA-676 during organogenesis caused dose related decreases in fetal weight in association with dose-related decreases in fetal skeletal ossification. Only one species was studied, thus the level of concern for Signal Strength I due to "cross species concordance" factor was unchanged. In addition to growth alterations, structural alterations and mortality were also observed. Concern is thus enhanced for the "multiplicity of effects" contributory element. Concern was also enhanced because effects were noted both in weight and ossification, perhaps reflecting adverse effects over a period of time.

An effect on fetal weight was noted prior to the appearance of maternal toxicity and concern was enhanced

based on this element under Signal Strength II. A clear dose dependent decrease in absolute weight of fetuses was observed. Thus, the level of concern was enhanced due to the "dose-response" element. Decreased fetal weight and delayed ossification are not considered rare events and concern was not adjusted for this element. Overall concern was increased +1 based on the Signal Strength II factor.

Endpoints not studied
Studies were not conducted to assess effects of CMA-676 on parturition, lactation or functional toxicities. The label should state that no information is available to assess risks of these toxicities in humans.

Overall conclusion
Based on net values (≥ 4) obtained from the table used for assignment of concern, there is a significant degree of concern in humans for impaired fertility, altered fetal growth, developmental mortality, and dysmorphogenesis from exposure to CMA-676 at the recommended dose.

OVERALL SUMMARY AND EVALUATION

CMA-676 is a chemotherapeutic immunoconjugate intended for the treatment of acute myeloid leukemia (AML). It is composed of a recombinant humanized monoclonal antibody linked to a potent antitumor antibiotic. In CMA-676, the recombinant engineered human monoclonal antibody, hP67.6, is covalently attached to N-acetyl-gamma calicheamicin dimethyl hydrazide (NAc-gamma calicheamicin DMH), a cytotoxic derivative of the calicheamicin family of antitumor antibiotics, by a bifunctional linker referred to as the AcBut linker. This linker is attached to the lysines of the antibody and also forms a hydrazone with the hydrazide of the NAc-gamma calicheamicin DMH. This hydrazone is presumably hydrolyzed in the acidic environment of the endosomes/lysosomes through which the antibody is routed after internalization. The hP67.6 antibody portion of CMA-676 binds specifically to the CD33 antigen. The CD33 antigen is expressed on the external surface of normal and leukemic myeloid cells, and leukemic blasts of 80% to 90% of AML patients, but not on normal hematopoietic cells. Therefore, CMA-676 only binds to leukemic cells and other cells of the myeloid lineage while sparing pluripotent stem cells (PSCs) and normal tissues. *In vitro* studies have demonstrated that human leukemia cells internalize the CD33 antigen after binding of an anti-CD33 antibody (mP67.6); internalization and catabolism of 38% of the CD33-antibody complex occurred within 4 hours. Such internalization of CMA-676 results in intracellular processing that releases the cytotoxic agent, NAc-gamma calicheamicin DMH, via hydrolysis of the hydrazone linkage. Calicheamicin γ_1^I (gamma) is the parent of a family of sequence-specific, minor-groove-binding, potent, DNA-damaging antibiotics that were originally isolated from a broth extract of a soil organism, *Micromonospora echinospora* ssp. *calichensis*. The methyl trisulfide of these "enediyne" antibiotics, as well as the disulfide of NAc-gamma calicheamicin DMH, react with reduced glutathione to form a glutathione disulfide. This disulfide exchange probably occurs after a calicheamicin derivative has left the acidic environment of the lysosomes. When calicheamicin derivatives undergo complete reduction and rearrangement, they eventually form a very reactive di-radical species. Deuterium-labeling experiments have shown that double-stranded DNA breaks are produced by the subsequent abstraction of very specific hydrogen atoms from deoxyribose rings on opposing strands of the DNA. Cell death is probably the direct result of such DNA damage. The end-product of the reaction of NAc-gamma calicheamicin derivatives with DNA is NAc-epsilon calicheamicin. This compound is at least 4000-fold less cytotoxic to the Raji Burkitt's lymphoma cell line *in vitro* than NAc-gamma calicheamicin.

CMA-676 demonstrated *in vitro* activity against the CD33-positive ($CD33^+$) HL-60 human promyelocytic leukemia cell line. CMA-676 was very potent, and demonstrated high selectivity for $CD33^+$ target cells relative to $CD33^-$ control cells. The unconjugated NAc-gamma calicheamicin DMH was much less cytotoxic to target cells than the conjugate but had comparable potency toward both the target and control cells lines. CMA-676 was 1000-fold more cytotoxic to HL-60 cells than a comparable conjugate made with a non-targeting control antibody, and the unconjugated hP67.6 antibody showed no cytotoxicity in this assay. The cytotoxic effects of CMA-676 were also investigated in HL-60, NOMO-1, N134, NKM-1 (all CD33 positive), K562 (weakly positive), and Daudi (CD33 negative) cells. Concentration-dependent, selective cytotoxicity was observed using flow cytometry to measure cell viability with the CD33 positive cell lines. Sensitive cells were temporally arrested at the G2/M phase of the cell

cycle, except for NKM- I cells. For the HL-60 cell line, morphological examination and DNA-fragmentation assays indicated that the primary cytotoxic effect of CMA-676 may be necrotic rather than apoptotic.

In vivo antitumor effects of CMA-676 were studied in an HL-60 xenograft tumor model. CMA-676 showed a dose-response effect with > 80 % inhibition of tumor growth at dosages between 0.8 and 2.4 mg/m^2 NAc-gamma calicheamicin DMH equivalents (36 to 108 mg protein/m^2). Significant increases in mortality occurred at the maximum studied dosage of 3.2 mg/m^2. At the lowest dosage of 0.8 mg/m^2, significant reduction of tumor growth was observed with no deaths, and 2 of 5 animals were tumor free at day 37. The dosages used in the xenograft model described above (36 to 108 mg protein/m^2) were much higher than the dosages used in clinical trials (0.25 to 9 mg protein/m^2) at least in part because the subcutaneously implanted HL-60 leukemia grows as a solid tumor. It is likely that significantly higher dosages were required in the xenograft model because solid tumors are targeted much less efficiently than naturally occurring leukemias.

Pharmacokinetics and Toxicokinetics

The pharmacokinetics of CMA-676 were characterized after administration of single and repeated IV doses in rats and monkeys. The tissue distribution and excretion of CMA-676 were examined after single IV dosing of tritiated CMA-676 in rats. Plasma concentrations of hP67.6 antibody, total calicheamicin derivatives, and unconjugated calicheamicin derivatives were used to characterize the pharmacokinetics of CMA-676. The pharmacokinetics of hP67.6 antibody and total calicheamicin derivatives after CMA-676 dosing in rats and monkeys were characterized by a very low systemic plasma clearance of 0.016 to 0.042 mL/min/kg, a very long $t_{1/2}$ of 100 to 200 hours, and a small volume of distribution of 0.1 to 1.5 L/kg. More than 98% of the circulating radioactivity related to the calicheamicin derivatives in plasma was associated with hP67.6 antibody for up to 120 hours after CMA-676 dosing. Results from the tissue distribution study indicated that CMA-676 was not rapidly distributed beyond the plasma compartment within the first 24 hrs. The major pathways for excreting CMA-676 appear to be biliary excretion and/or gastrointestinal secretion. The pharmacokinetics of unconjugated calicheamicin derivatives after CMA-676 dosing could not be characterized because plasma concentrations of the unconjugated derivatives were generally below the assay limit of quantitation in rats and monkeys. The low plasma concentrations of unconjugated calicheamicin derivatives are most likely due to a slow formation rate of the unconjugated derivatives from CMA-676 and a rapid clearance rate of the unconjugated derivatives. Results from single-dose IV pharmacokinetic studies of NAc-gamma calicheamicin DMH and NAc-epsilon calicheamicin in rats and dogs indicated that the unconjugated calicheamicin derivatives are eliminated at rapid clearance rates close to or exceeding the hepatic plasma flow rates of approximately 27 mL/min/kg in rats and approximately 15 mL/min/kg in dogs.

Comparison of Systemic Exposures Following Repeated Dose Toxicity Studies with CMA-676 at MTDs

Parameters	Rats	Monkeys	Human
HNSTD or MTD (mg protein/m^2)	8.4	22.1	9.0
hP67.6 Antibody $AUC_{0-\infty}$ (µg eq.h/ml)	1420	5250	239
Total Calicheamicin Derivatives $AUC_{0-\infty}$ (µg eq.h/ml)	9.2	38.0	5.2
Unconjugated Calicheamicin Derivatives C_{max} (ng eq/ml)	17.6	11.4	5.0

Mean systemic exposures (based on AUCs of hP67.6 antibody) in rats and monkeys at HNSTD were 5.9 and 22-fold higher, respectively, than that of AML patients given 9 mg protein/m^2. For total calicheamicin derivatives, the mean systemic exposures (based on AUCs) at HNSTD in rats and monkeys were 1.8 and 7.3 fold higher, respectively, than that of AML patients. Based on C_{max} values of unconjugated calicheamicin derivatives, exposures at HNSTDs in rats and monkeys were 3.5 and 2.3 fold higher, respectively, than that of AML patients. Since

ELISA methods were used, it was only possible to quantitate hP67.6 antibody, total calicheamicin derivatives, and unconjugated calicheamicin derivatives in plasma, whereas no information is available for the intact CMA-676. Thus, the data presented in the table above is not adequate for assessment of exposure and comparison across species.

The metabolic fate of NAc-gamma calicheamicin DMH and NAc-gamma calicheamicin DMH AcBut was examined *in vitro* in human liver microsomes and cytosol, and of NAc-gamma calicheamicin DMH in HL-60 promyelocytic leukemia cells. Many metabolites of the calicheamicin derivatives were found after incubation in human liver microsomes and cytosol. The biotransformation pathways in microsomes were oxygenation and demethylation, while the formation of NAc-epsilon calicheamicin and its derivatives appeared to be the major pathways in cytosol. Several metabolites, including NAc-epsilon calicheamicin and its isomer, were produced during incubation with the HL-60 leukemia cells. Common metabolites were found in both liver and leukemia cell preparations, suggesting that the metabolism of the calicheamicin derivatives may not be cell specific. The detection of NAc-epsilon calicheamicin and its derivatives in cells supports the hypothesis that the reactive diradical species of NAc-epsilon calicheamicin probably is formed via a glutathione-dependent reduction of the disulfide bond of NAc-gamma calicheamicin DMH within cells.

Toxicology

When given weekly for 6 doses, the CMA-676 was not lethal to rats up to 7.2 mg/m^2 nor in monkeys up to 21.6 mg/m^2. In rats, gross pathologic changes in the 7.2 mg/m^2 group which did not resolve after 4 weeks of recovery were small testes and pale kidneys. Histopathologic changes at 7.2 mg/m^2 that worsened in the liver during recovery were karyocytomegaly and oval cell/bile duct proliferation. Marked changes in the histopathology of the testes, atrophy of the mammary glands, and slight changes in the kidney were also noted at this dose, that did not resolve during recovery. These changes were consistent with clinical chemistry and hematologic changes indicative of hepato, renal, and hematopoietic toxicities resulting from hP67.6 conjugate administration. In monkeys given multiple doses of hP67.6 conjugate, histopathologic changes at 21.6 mg/m^2 still present after the recovery period were slight tubular basophilia and moderate amount of eosinophilic material in the kidney, slight brown pigmentation of Kupffer cells and moderate single cell hepatocyte necrosis, and atrophy of the lymphoreticular system. These gross and histopathologic changes were consistent with clinical chemistry and hematology changes indicative of mild hepato, renal, and hematopoietic toxicities associated with the drug product. The hepatotoxicity was consistent with the distribution of CMA-676 to the liver and the predominant biliary excretion of this drug.

Reproductive Toxicity

Daily treatment of pregnant rats with CMA-676 (0.01 to 0.06 mg/kg/day) during organogenesis caused dose related decreases in fetal weight in association with dose-related decreases in fetal skeletal ossification. Doses of 0.060 mg/kg/day produced increased embryo-fetal mortality (increased numbers of early and late resorptions and decreased numbers of live fetuses per litter). Five of 179 fetuses (3% overall incidence) from 4 of 24 litters had digital malformations (ectrodactyly, brachydactyly) in one or both hind feet. Similar digital malformations occurred in 22% of examined fetuses at a higher dosage of 0.075 mg/kg in the dose-range finding study. A single fetus in the 0.060 mg/kg group had a short tail and no other gross external or skeletal malformation. Unlike the digital malformations, short tail was not observed in the dose-range finding study at the higher dosage of 0.075 mg/kg. Other visceral, and skeletal alterations at the dose of 0.060 mg/kg included absence of the aortic arch, wavy ribs, anomalies of the long bones in the forelimb(s) (short/thick humerus, misshapen radius, misshapen ulna, and short/thick ulna), misshapen scapula, absence of vertebral centrum, and fused sternebrae. Integration of all available non-clinical data indicate significant concern for adverse effects of CM-676 on human fertility and fetal development (including growth, survival, and morphogenesis).

Genetic Toxicology

CMA-676 was clastogenic in an *in vivo* mouse micronucleus assay and was consistent with the induction of DNA breaks by the calicheamicins and other antitumor antibiotics.

RECOMMENDATION The pharmacology/toxicology data supports approval of CMA-676 for the treatment of patients with CD33 positive acute myeloid leukemia in first relapse who are 60 years of age or older. Recommended revisions to the labeling are noted in a separate review.

a) Comments for further studies: none

b) Points discussed with Medical Officer: none

Draft studies: n/a

Draft Letter to the Sponsor: none

/S/

Sandip K. Roy, Ph.D.
Pharmacologist/Toxicologist

4/14/2000
Date

/S/

Paul Andrews, Ph.D.
Pharmacology Team Leader

4/14/2000
Date

Original NDA
c.c. /Division File
/PAndrews
/PBross
/SBradley
/SRoy

Bradley

APR 19 2000

Division of Oncology Drug Products, HFD-150

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Review No. 2, Labeling Review

Key words: CMA-676, calicheamicin, $CD33^+$ antigen

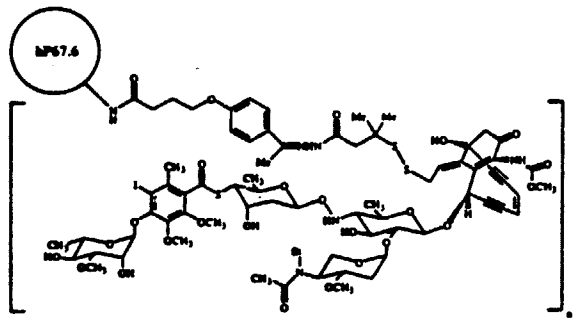
NDA No. 21-174
Serial No(s). 000 **Type:** NDA **Letter Dated:** 10/29/1999 **Received by CDR:** 10/29/1999

Information to be conveyed to the sponsor: No
Reviewer: Sandip K. Roy, Ph.D.
Review Completion Date: 4/19/2000
Sponsor: Wyeth-Ayerst Research
Philadelphia, PA
Manufacturer: Same as above
Drug:

Code Name: CMA-676
Generic Name: Gemtuzumab Ozogamicin
Trade Name: Mylotarg
Chemical Name: hP67.6 conjugate; 555,201;
hP67.6-NAc-γ_1^I-calicheamicin DMH AcBut conjugate

Other Relevant Names:		
	184,538	NAc-γ-calicheamicin DMH
	191,305	NAc-γ-calicheamicin DMH AcBut
	181,441	γ_1^I-calicheamicin
	555,001	hP67.6 "naked" antibody
	181,287	ε-calicheamicin ("triggered form")
	190,396	NAc-ε-calicheamicin

CAS Registry Number: not known
Molecular formula/weight: not known
Structure: The MoAb is a humanized murine monoclonal antibody to human CD33.



n = 0, 1, 2, 3...; average n = 1.9 to 3.1

Related INDs, NDAs, DMFs: IND [redacted]
Drug Class: Antineoplastic
Proposed Indication: Treatment of patients with CD33 positive acute myeloid leukemia in relapse

Clinical Formulation: Each vial contains lyophilized powder containing 5 mg of drug conjugate (protein equivalent). Also contains dextran 40, sucrose, sodium chloride, monobasic and dibasic sodium phosphate. Reconstituted with 5 ml Sterile Water for Injection, USP.

Route of administration and dosage form: IV infusion

Proposed Clinical Dose: The recommended dose of TRADENAME is 9 mg/m^2, administered as a 2-hr intravenous infusion

Previous Review(s), Date(s), and Reviewer(s): IND [redacted] Review #1, 12/29/94, Paul A. Andrews, Ph.D.; IND [redacted] Review # 2, 9/5/96, Paul A. Andrews, Ph.D.; NDA 21-174 Review #1, Sandip K. Roy, Ph.D.

Labeling Comments:

This review contains pharmacology/toxicology comments on the sponsor's proposed label for Mylotarg (TRADENAME in label). The following changes are recommended.

● The **DESCRIPTION** section was extensively revised during the first team labeling meeting. The current version is acceptable.

● Make changes in the **"CLINICAL PHARMACOLOGY"** section as follows:

CLINICAL PHARMACOLOGY
General

/S/ 4/19/2000
Sandip K. Roy, Ph.D. Date
Pharmacologist/Toxicologist

/S/ 4/19/2000
Paul Andrews, Ph.D. Date
Pharmacology Team Leader

Original NDA
Cc: /Division File
/PAndrews
/PBross
/SBradley
/SRoy